ROLE OF CORNEAL COLLAGEN CROSS-LINKING WITH RIBOFLAVIN IN KERATOCONUS

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA

M.S. DEGREE EXAMINATION
BRANCH – III OPHTHALMOLOGY

APRIL – 2014

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The following scientific paper, submitted to *med ej*, has been approved.

Title: 'Safety and efficacy of Scleral fixated Intraocular lenses in Paediatric population.'

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CERTIFICATE

This is to certify that the dissertation entitled **"ROLE OF CORNEAL COLLAGEN CROSS-LINKING WITH RIBOFLAVIN IN KERATOCONUS"** is a bonafide work done by **Dr.BHAVATHARINI M**, Postgraduate student in M.S. (Ophthalmology) during April 2011 to March 2014, under our direct supervision and guidance, at our Institute, in partial fulfillment for the award of M.S. Degree in Ophthalmology of the Tamilnadu Dr.M.G.R. Medical University, Chennai.

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ACKNOWLEDGEMENTS

I sincerely express my gratitude to Professor and Director, **Dr. C. A. Nelson Jesudasan, MS., DOMS., FRCS (Edin&Glas**), for providing me with all the necessary infrastructure and for creating an environment which facilitates research of this kind.

I would like to thank **Prof. Dr. Pragya Parmar MS.,** my guide, for her constant support, meticulous care and valuable guidance at each step of making of my dissertation.

I would like to thank **Prof. Dr. Rajamohan , MS, DO.,** my co-guide for his blessings and for being an everlasting source of inspiration.

I express my heartfelt gratitude to **Prof. Dr. Amjad Salman, MS.,** without whose excellent guidance, valuable suggestions and encouragement, it would not have been possible for me to complete this dissertation.

I owe my gratitude to **Prof. Dr. A. Philip Thomas, MD., Ph.D.,** who has gone through each and every word of my dissertation and has provided scholarly supervision for carrying out this work.

I acknowledge the timely help and motivation provided by **Dr. Sowjanya MS, DO.,** which has helped me immensely in completing this study.

I convey my special thanks to my friends for always being there in my time of need.

I thank all the staff of the department of Cornea for their co-operation and technical assistance.

I express my sincere thanks to my patients who consented and participated whole heartedly in this study.

Last but not the least, the most important pillars of support in my life, my parents and my brother who have been with me through all the ups and downs. Acknowledgement is just an expression, Thank you.

And above all, I thank Almighty God for having made all this possible.

ROLE OF CORNEAL COLLAGEN CROSS-LINKING WITH RIBOFLAVIN IN KERATOCONUS

ABSTRACT

AIM:

To evaluate the safety and efficacy of corneal collagen cross-linking with riboflavin (C3R) in keratoconus and its role in preventing the disease progression.

MATERIALS AND METHODS:

This was a prospective interventional study conducted on patients with progressive keratoconus who presented to the Cornea Clinic, at a tertiary eye care hospital in Tamilnadu over a 20 month period (April 2011 to November 2012) with a follow-up of one year were enrolled. The outcome measures such as uncorrected visual acuity, best corrected visual acuity, spherical equivalent, cylindrical error, keratometry values (Kmax, Kmin, Kavg), and the rate of development of complications if any, were analysed at one month, 6 months and at one year post-C3R.

RESULTS:

Fifty eyes of 38 patients comprising 20 males (53%) and 18 females (47%) with progressive keratoconus with a mean age of 19.2 ± 4.5 years (range: 11-25 years), were included. Visual acuity improved significantly from the baseline till one year of follow-up in all treated eyes. ($p<0.0001$).There was gradual reduction in the keratometry readings throughout the follow-up period in a significant manner ($p<0.0001$). The demographic factors such as age, gender and other pre-operative indices like corneal thickness and the average keratometry value did not influence the outcome of this procedure. Complications in the form of corneal ulcer developed in two (4%) of the 50 eyes

in this study, and this was attributed to the use of bandage contact lens post-procedure.

CONCLUSION:

Corneal collagen cross-linking with riboflavin is a simple, safe and an effective modality of treatment for progressive keratoconus with good success rate and minimal incidence of complications. However, longer follow-up is required for assessing the durability of this procedure and its long term side effects.

Keywords: C3R, corneal collagen cross-linking, keratoconus.

CONTENTS

Introduction

INTRODUCTION

Corneal ectasia refers to a group of disorders affecting the shape of the cornea and includes entities such as keratoconus, keratoglobus, pellucid marginal degeneration and iatrogenic causes such as progressive post-lasik keratectasia (PPLK). In ectasia, corneal thinning occurs which leads to bulging of the cornea, resulting in regular or irregular astigmatism. This astigmatism causes reduced visual acuity in these patients.

Ectasias are usually bilateral in nature, asymmetric and non-inflammatory. The incidence of ectatic disorders of the cornea is higher in the young population. Keratoconus is a common ectatic disorder affecting the eye and is one of the major causes of reduced vision in this population.

Keratoconus is a bilateral, asymmetric, non-inflammatory, progressive ectasia that causes steepening of the cornea. The incidence of this condition in the population is 1 in 2000 and it especially affects individuals in their early teens. (Rabinowitz *et al.*, 1998)[1.] The cornea assumes a conical shape most typically inferior to the center, because of the ectasia, which leads to an off-center apex, causing irregular astigmatism.

Histopathologically, keratoconus exhibits the triad of thinning of the corneal stroma, breaks in Bowman's membrane and deposition of iron in the basal layer of the corneal epithelium.

Current therapeutic options for keratoconus includes spectacle correction for myopic astigmatism in early stages, rigid contact lenses, scleral contact lenses and customized lens like Rose K lens™ (Menicon Co, Ltd, Nagoya, Japan), intra stromal corneal ring segments (INTACS), and lamellar and penetrating keratoplasty.

Recently, after a detailed study of the pathogenic mechanisms involved in keratectasia, a new technique has emerged, namely corneal collagen cross-linking using riboflavin (C3R). The major advantage of this technique over all other modalities is that C3R arrests progression of the ectasia. (Wollensak *et al.*, 2003)[2]

C3R is also referred as CCL or CXL. This technique was described by Theo Seiler, Eberhard Spoerl (Spoerl E *et al.*, 1998)[3] and Wollensak [(2)] at the University of Dresden, Germany. Further studies have been conducted by Caporossi and Roberto Pinelli in Italy and also Brian Boxer in the United States of America (USA).This technique increases the mechanical and chemical stability of the corneal tissue by creating

additional chemical covalent bonds in the corneal stroma by means of highly localized photopolymerization. (Wollensak *et al.*, 2006)[4]

C3R involves the sequential use of riboflavin (vitamin B2) and ultraviolet light in the A spectrum (UV-A) to induce collagen crosslinking in the corneal stroma, thus enhancing its biomechanical rigidity. This leads to either slowing down or total arrest of the progression of ectasia [(2,4)]. Custom made riboflavin drops used in a concentration of 0.1% act as the photosensitiser ; they are non-toxic and soluble in nature. UV-A, having a wavelength of 370 nm, is used for exciting the already sensitized cornea, because this particular wavelength is strongly absorbed and also protects the deeper layers of the eye ; also, the absorption peak of riboflavin is achieved at this wavelength.

Riboflavin, upon excitation is converted into a triplet state, which gives rise to reactive oxygen species (ROS). These ROS react with the chemically covalent bonds, bridging the amino acid groups of collagen fibrils in a Type II photochemical reaction [(4)].

The procedure increases corneal rigidity by 328.9% (Wollensak *et al.*, 2003)[5]. This is because of an increase in the diameter of the collagen fibrils mainly due to inter-fibrillar and intra-fibrillar covalent bond

formation, finally resulting in a more compact and strong cornea which is resistant to deformation and ectasia.

Current indications of C3R include progressive keratoconus, pellucid marginal degeneration and iatrogenic post refractive or post-LASIK ectasia. Even though it slows the progression of keratoconus, it cannot be used in corneas thinner than 400 microns to prevent damage to endothelium and the deeper structures of eye (Spoerl *et al.*, 2007)[6]; and also in previously scarred corneas.

Advantages of C3R include the halt in progression of the ectasia [2,4], which is not provided by any other modality. It is also simple to perform, easier to learn and requires a shorter rehabilitation period than other therapeutic modalities. The important point is that C3R, being a totally extraocular procedure, is devoid of all the complications associated with other procedures such as keratoplasty.

A putative 'con' of this procedure is its durability is unknown; also, it may have to be repeated (rarely) to treat a recurrent ectatic condition. Mild stromal haze is seen in a few patients which resolves within a few months. The major drawback of this procedure is that it cannot be used in extremely thin corneas (less than 400 micron thick) and corneas that have been scarred as a sequel to acute hydrops. Hypotonic riboflavin can be

used in corneas less than 400 micron thick to swell the stroma; C3R can be performed after pachymetry if a value of greater than 400 microns is achieved after hypotonic riboflavin. (Hafezi F *et al.*, 2009)[7]

Various modifications of the basic procedure have been described. They include the original Seiler method [2,4], Caporossi's technique[8], Kanellopolus intralase technique[9], Sanchez Leon modified method in post-LASIK ectasia, phototherapeutic keratectomy(PTK) cross-linking and also simultaneous topography guided photo refractive keratectomy (PRK) and cross-linking.

Complications associated with this technique have been few and infrequently reported. They include formation of opaque patches over time at the stromal level; occurrence of infection due to epithelial scraping or the use of contaminated riboflavin solution or bandage contact lens (BCL) induced infection; corneal endothelial toxicity secondary to the use of high fluence riboflavin or high fluence of the UV-A light especially in thinner corneas. The last complication can be easily avoided by proper calculation of the intensity of UV light and adequate saturation of riboflavin drops in corneal stroma which can be seen on slit lamp examination (yellow flare in anterior chamber under the blue filter). Riboflavin prevents UV-A absorption beyond 300 microns and it reduces the chance of endothelial

damage and potential recurrence of corneal ectasia. A new group of complications has emerged after the introduction of combined use of C3R and PRK; these include persistent epithelial defect and permanent scarring of the cornea.

C3R is a new technique and it is being tried in various corneal pathologies, alone as well as in combination with other modalities. Apart from the current indications, which have been previously mentioned above, C3R is now also being used in combination with intra stromal corneal ring segments (INTACS) for treatment of severe keratoconus. It is also being done before PRK and to delay the time of corneal transplantation.

The other pathologies for which C3R has been tried and is under research include severe corneal edema, pseudophakic bullous keratopathy, corneal melting and recalcitrant infections causing corneal ulceration. To avoid infection, a new “no touch technique” using excimer laser episcrape is being used, instead of manual debridement of the epithelium.

Overall C3R is a simple, safe and effective new technique which is attracting a lot of attention in the treatment of corneal ectasia and also is under study for treatment of various other corneal pathologies. The benefits of this technique clearly outweigh the drawbacks and C3R may

emerge as the standard treatment modality for corneal ectatic disorders especially progressive keratoconus.

Hitherto, the most successful modality of treating early and moderate cases of keratoconus have been gas-permeable contact lenses, as they have the ability to correct irregular astigmatism of relatively higher levels, with substantial improvement in visual acuity.(Romero-Jimenez *et al.*, 2013)[10].When this option is not available, about 12% of individuals with keratoconus require penetrating keratoplasty surgery. (Gorden *et al.*, 2006)[11].

More recently, C3R has been developed and used to treat keratoconus (Wollensak *et al.*, Caporossi *et al.*)[2,8]. However, these studies were done on small number of patients, and the duration of the condition was variable.

Hence, in the current investigation, an attempt has been made to study the efficacy of corneal collagen cross-linking in patients with progressive keratoconus in the Indian population.

Aim of the Study

AIM OF THE STUDY

a) To evaluate the efficacy of corneal collagen cross-linking with riboflavin (C3R) in keratoconus by studying outcome in terms of uncorrected and best corrected visual acuity; spherical equivalent, cylinder value, and corneal topography.

b) To assess the role of C3R in preventing the progression of keratoconus.

c) To assess the safety of C3R by noting the frequency, severity and types of adverse events occurring during and after the procedure.

Review of Literature

REVIEW OF LITERATURE

Keratoconus is a bilateral, non-inflammatory corneal disorder that leads to steepening of the inferior paracentral cornea with irregular astigmatism. (Krachmer JH *et al.*, 1984)[12].These patients present with complaints of decreased visual acuity, glare, and photophobia.

Clinical signs of keratoconus include Munson's sign (protrusion of the lower lid on downward gaze); and Rizzuti's sign (conical reflection of the nasal cornea when light is shone temporally). Slit-lamp examination of corneas of these patients shows inferior paracentral corneal thinning, presence of an ectatic cone, inferior corneal steepening, vertical stress lines in the posterior stroma (Vogt's striae), iron deposition in the basal epithelium (Fleischer ring), and linear scar as a result of break in the Bowman's layer.

Retinoscopy of eyes with keratoconus reveals a scissoring reflex and the presence of Charleaux oil reflex (bright reflex from the conical area surrounded by a dark circular shadow produced by the ectasia.)

The pathology of keratoconus include three basic mechanisms, namely thinning of the stroma, breaks in the Bowman's membrane and iron deposition in the basal layer of epithelium.

Physiologically, in keratoconus, there is a reduction in the corneal hysteresis (that is cornea is less capable of absorbing energy) and also the corneal resistance factor (that is corneal rigidity is lowered) (Ortiz D *et al.*, 2007).[13]

Corneal ectasia is difficult to detect clinically, especially in early stages. Pachymetry is useful for detection of keratoconus since it shows the relationship of the apical, central and thinnest part of the cornea. A diagnostic tool for corneal ectasia is topography.

Corneal topography provides useful and accurate information with regards to the position of the ectasia; it also permits detection of progression and is helpful in detection of early cases. The posterior corneal shape is used mainly for early recognition of this pathologic condition.

On topography, keratoconus can present either as a central symmetric but lopsided or "lazy eight" bow tie with skewed radial axes or

as an asymmetric bow tie with or without skewing. (Rabinowitz and Rasheed.,1999)[14].

Rabinowitz has suggested diagnosis on the basis of presence of keratometry of more than 47.20 D, steepening of inferior cornea as compared with superior cornea of more than 1.2 D, skewing of radial axis of astigmatism by more than 21 degree. (Rabinowitz YS *et al.*,1995)[15.]

An important characteristic of keratoconus is that its progression is uneven between the two eyes. In familial keratoconus, a 'J' pattern or an 'inverted J' pattern may be noted. (Levy *et al.*,2004)[16.]

To differentiate early ectatic changes or "forme-fruste" changes from more stable forms, Maeda *et al.*, (1997) [17] demonstrated a method of classifying corneal maps; this method was found to be more sensitive and specific than looking for elevated Sim-K readings and infero-superior asymmetry (I-S) values.

There are several commercially available instruments which may serve a diagnostic and prognostic role in corneal ectasia. The Magellan Mapper ™ (Nidek, Gamagori, Japan) features a software that detects

various corneal diseases by using a neural network application.(Klyce D *et al.*, 2005)[18]

The Orbscan™ corneal topography system (Bausch and Lomb, Rochester, NY, USA) uses a placido device to determine the corneal curvature. It takes 40 slit sections of the cornea during two scans, and the anterior and posterior corneal height profiles are reconstructed from these sections. This system provides accurate information regarding the morphology and topographic changes related to keratoconus. This system is able to suggest a risk for ectasia if there is, a variance of more than 1.00D in astigmatism between two eyes, keratometric or corneal steepness on the mean power map, or irregularity at 3 mm to 5 mm of the central cornea; a risk for ectasia is also suggested if the posterior surface float is greater than 0.05 mm or if the thinnest area of corneal thickness is more than 20 microns thinner than the thickness of the central cornea; two abnormal maps may indicate early keratoconus or corneal ectasia.(Karpecki *et al.*, 2006)[19]

The Pentacam™ (Oculus, Wetzlar, Germany) is a rotating Scheimpflug camera with a higher depth of focus. This instrument assesses the anterior chamber of the eye, the topographic corneal thickness, corneal

curvature, anterior chamber angle and volume and height upto 25, 000 true elevation points. When using this instrument, anterior elevation differences less than +12 microns are considered normal, anterior elevation differences greater than +15 microns are considered indicative of keratoconus, and anterior elevation differences between +12 to +15 microns are considered suspicious. Similar figures, upto +5 microns higher, indicate posterior elevation. (Maus M *et al.*, 2006)[20]

The Galilei ™ dual Scheimpflug analyser (Ziemer Ophthalmic systems AG, Port, Switzerland) is an instrument that, has a placido disc function; this instrument combines the slit and the placido data in displaying corneal topography.(Klyce D *et al.*, 2009)[21]

The Reichert Ocular Response Analyzer™ (Reichert Inc, Depew, NY, USA) provides measurement of corneal hysteresis (CH) and the corneal resistance factor (CRF). It can also provide a repeatable, Goldmann tonometer-correlated intraocular pressure measurement. It is believed that CH and CRF might be useful as diagnostic tools for determining who might be at risk for developing post-refractive ectasia (Kirwan *et al.*, 2008)[22]; (Ortiz D *et al.*, 2007)[13].

Maeda *et al.*, (2002)[23] suggested that wavefront aberrometers may provide additional clues for the detection of early corneal ectasia; an increase in the total higher order aberrations was noted in keratoconus and was attributed to the corneal shape. Coma like aberrations were common in keratoconus eyes.

Therapeutic options currently available for ectatic disorders include the use of rigid contact lenses, specialized contact lens, INTACS, cross-linking with riboflavin, and lamellar or penetrating keratoplasty.

Cross-linking is a method that is widely used in the polymer industry to harden materials, and also in bioengineering to stabilize tissues. The most extensively studied and frequently used method as far as the cornea is concerned is UV-A/riboflavin collagen cross-linking. This method utilizes UV-A at 370 nm to activate riboflavin which is excited to a triplet state. This further generates reactive oxygen species (ROS), which induce inter-fibrillar and intra-fibrillar covalent bonds in the stromal collagen [(4)].

In animal studies, collagen cross-linking with riboflavin/UV-A treatment has been found to induce a significant increase in corneal rigidity by approximately 70% [5].

In humans, ultraviolet cross-linking treatment appears to be able to halt progression of corneal ectasia in keratoconus and other condition [2]. This effect of halting or even reversing the progression of the ectasia is the major advantage of C3R over other treatment modalities of treatment of corneal ectasias.

Cross-linking brings about biomechanical, thermochemical, morphological and biochemical effects, and also exhibits effects on keratocytes, collagenase existence and hydration behaviour in the cornea.

The tensile strength of the cornea is decreased in keratoconus. Biomechanical stress strain measurements in human corneas showed an increase in corneal rigidity of 328.9% and an increase in Young's modulus by the factor of 4.5 after cross linking[5]. The cross-linking effect is maximal in the anterior 300 microns of the cornea[5].

The maximal hydrothermal shrinkage temperature was found to be 75 degree for cross linked porcine corneas and 70 degree for untreated

controls; this effect is more pronounced in the anterior stroma of the cornea (Spoerl E *et al.*, 2004)[24].

Following cross-linking, the collagen fiber diameter was found to be increased by 12.2% in the anterior stroma and by 4.6% in the posterior stroma in rabbit eyes; this was hypothesised to be because of the induced cross-links, pushing the collagen polypeptide chains apart, resulting in increased intermolecular spacing. An increase in collagen fiber diameter and corneal rigidity due to collagen cross-linking has also been observed in diabetes mellitus and aging. (Wollensak *et al.*, 2004)[25].

Cross-linking was found to increase resistance against collagenase digestion in porcine eyes; this effect being stronger in the anterior part of the cornea. Such increased resistance to collagenase digestion possibly plays an important role in ameliorating keratoconus, since collagenase activity is increased in keratoconus. In the tear samples from keratoconus patients, collagenase metabolites were found to be 2.5 times higher than those in normals. (Spoerl *et al.*, 2004)[26].

Gel electrophoresis of cross-linked porcine corneas, revealed an additional intensely staining polymer band in the stacking gel that was

resistant to mercaptoethanol, heat and pepsin treatment. The presence of this polymer band is consistent with the morphologic correlate of an increased fiber diameter after cross-linking treatment. The chemical stability of cross-linked corneal fibers supports the hopes of a long-term effect of the new treatment.(Wollensak *et al.*, 2008)[27].

When cross-linked porcine eyes were examined by biomicroscopy, optical coherence tomography (OCT) and light microscopy, a lower degree of edema was found in the anterior stroma, confirming previous findings that the cross-linking effect is strongest in the anterior part of the corneal stroma. However, cross-linked corneas did not induce a specific signal on OCT, suggesting that OCT is not suitable as a method of clinical control of the cross-linking effect. (Wollensak *et al.*, 2007)[28].

Cross-linking has also been found to cause keratocyte apoptosis on anterior corneal stroma of rabbit eyes. Keratocyte apoptosis is sometimes reflected clinically by a transient mild corneal edema.

Heidelberg Retinal Tomograph II ™(Heidelberg, Germany) in vivo confocal microscopy in human eyes with keratoconus also showed rarefaction of keratocytes in the anterior and intermediate stroma

associated with stromal edema (spongy or honeycomb-like) immediately after cross-linking treatment. Three months after cross-linking, keratocyte re-population and disappearance of edema was observed. Six months after the cross-linking, keratocyte re-population was complete.(Mazotta *et al.*, 2007)[29].

Keratocyte apoptosis is not only observed after cross-linking but also in corneal abrasions, herpes virus keratitis and keratoconus and following procedures such as photorefractive keratectomy, LASIK and Epikeratophakia.(Wollensak *et al.*, 2004)[30].

Wollensak *et al.* (2003) [2] are credited with the first clinical study that showed that corneal cross-linking (exposure to UV-A light at 3.0mW/cm2 and riboflavin 0.1% for 30 minutes) was able to stop progressive keratoconus in all 23 eyes of 22 patients; these patients ranged from 13 to 58 years in age with the average age being 34.7 ± 11.9 years. Steep keratometry values reduced from 2.01 Diopters in 70% of the eyes with a refractive correction of 1.14 diopters. Over an average follow-up time of 23 months, no scarring in the cornea, no lenticular opacities and no endothelial cell loss was seen, while intraocular pressure did not change after the procedure.

In a subsequent study, these authors found that C3R is able to stabilize the cornea in keratoconus and may diminish the need for corneal transplant [(4)].

Five year follow-up results to this pioneering study have been published in a subsequent review of cross-linking by Wollensak [(4)]. In 150 eyes treated, 60 eyes had a 5-year follow-up and no progression of keratoconus was seen in any of these patients. In 31 eyes (52%), a reduction in keratometry of 2.87 diopters was seen with best spectacle corrected visual acuity (BSCVA) improving by 1.4 lines.

Caporossi *et al.* (2006)[8] in their prospective study conducted in 10 eyes with bilateral keratoconus in Italy reported reduction in mean keratometry reading confirmed by topography, and improvement in visual acuity following C3R.

These authors also conducted a long term trial in Italy, widely known as the Siena Eye Cross Study on 363 eyes with progressive keratoconus with a mean follow-up of 52.4 months found long term stability of keratoconus with improvement of visual acuity and no side effects. (Caporossi *et al.*, 2010)[31]

Raiskup-Wolf *et al.* (2008)[32] following their long-term study on 480 eyes of 272 individuals with keratoconus in Germany, concluded that there is a long-term stabilization and improvement after collagen cross-linking; they thus felt that collagen cross-linking is an effective therapeutical option for progressive keratoconus.

Goldich *et al.* (2012)[33] in Israel reported visual acuity improvement and stabilisation of keratoconus at two years following C3R, in their prospective study on fourteen patients. They also observed no change in corneal thickness, endothelial cell density and foveal thickness suggesting the safety of this procedure.

In a prospective comparative study on 55 eyes of 39 patients by Muriel *et al.*(2013)[34] in France, showed stabilisation of keratoconus with no side effects, at the end of three year following C3R.

Viswanathan *et al.* (2013)[35], concluded that corneal collagen cross-linking using riboflavin and ultraviolet-A is effective as a therapeutic option in cases of progressive keratoconus by reducing the corneal curvature and improving the visual acuity in these patients; their study was performed in 35 patients.

Vinay Agrawal *et al.*(2009)[36] in their retrospective analysis of effect of C3R on 37 Indian eyes with one year follow-up reported reduction in the K value in 66% of eyes by a mean of 2.73D and in 22% of the eyes it remained stable. Thus they suggested C3R as an effective treatment for progressive keratoconus.

In another retrospective study of 28 eyes with advanced keratoconus with maximum K value of more than 55D preoperatively showed improvement in 27 eyes following C3R at last visit. Thus they concluded C3R helps in preserving visual acuity and delaying keratoplasty in patients with advanced progressive keratoconus. (Ivarsen *et al.*, 2013)[37]

The Sienna CXL Paediatrics study in 152 patients less than 18 years of age observed rapid and stable functional recovery in terms of UCVA, BCVA and coma values in 80% of the cases without any complications. Hence they suggested the elective use of C3R for treating progressive keratocnus in paediatric population. (Caporossi *et al.*, 2012)[38]

Lamy *et al.* (2013)[39] in Brazil showed improvement in contrast sensitivity in all 68 eyes with progressive keratoconus treated with C3R at the end of two years.

Hafezi *et al.* (2009)[7] in a study on 20 individuals in Switzerland, concluded that the preoperative swelling of the cornea safely broadens the spectrum of C3R indications to thin corneas that would otherwise not be eligible for treatment.

Caporossi *et al.*(2012)[40] in 10 patients noted that in vivo confocal analysis of corneal modifications induced by trans-epithelial corneal collagen cross-linking (TE C3R) showed a limited apoptotic effect of this treatment, about one-third of classic epi-off crosslinking procedure. The TE C3R respected sub-basal and anterior stroma nerve fibers, and was thus safe for corneal endothelium.

Caporrosi *et al.*(2013)[41] in a prospective study, reported unstable functional recovery in 26 patients after trans-epithelial CXL. Re-treatment was required in 50% of these patients with epi-off CXL.

C3R has gained mass appeal and is likely to become the standard treatment modality for early and moderately advanced progressive keratoconus. However, individuals with stromal scarring, corneal thickness of less than 400 microns at the thinnest point are poor candidates for C3R.

C3R is also a viable option in progressive post-LASIK keratectasia, which hitherto, had the only option of a custom designed contact lens or a corneal transplantation. Here also a minimum thickness of 400 microns in the thinnest point of the cornea is a limiting factor. The process of swelling the cornea to 400 microns and then harnessing C3R is the way to proceed. The hypoosmolar solution of 0.5% Riboflavin (diluted in BSS in a 1:4 concentration) is applied till the cornea swells to a thickness of 400 microns. Also one should not swell a keratoconic cornea more than 80 microns as these corneas have a different swelling pattern.

Crosslinking has shown favorable outcomes in eyes with pellucid marginal degeneration. However, a larger 11 mm area should be exposed as of against the usual 9 mm advocated. A merocoel protection ring is advised to protect the limbal stem cells and also an eccentric corneal light exposure is advised.

Nowdays, C3R is being combined with intrastromal corneal ring segments (INTACS) in an attempt to flatten the cornea. However it does not solve the problem of weakening of collagen. INTACS acts as an additive measure to flatten the cornea. Another mechanism proposed is

that, INTACS cause pooling of the riboflavin in the area of its segment which has the maximum cross-linking effect.

Coskunseven *et al.* (2009)[42] in a study on individuals with keratoconus in Turkey, observed that implantation of ICRS followed by C3R resulted in greater improvement of keratoconus.

Kilic *et al.* (2012)[43] in Turkey, observed that combined ICRS and C3R treatment with intracorneal riboflavin injection was effective in 131 keratoconic eyes. Intracorneal riboflavin injection into the tunnel was safe. The authors felt that intracorneal riboflavin injection might provide more penetration without epithelial removal.

The pre-treatment of corneas with C3R before PRK by a couple of months is a possible detour, it gives a window period of better visual acuity till the eventual corneal transplant is planned.

C3R treatment improves cross-linking and tightens the peripheral incisions of the radial keratotomy (RK) which leads to a more stable cornea. This halts the progressive flattening and the induced hyperopia post-RK.

Other applications of C3R are being studied in cases of pseudophakic bullous keratopathy where the corneas need to be initially deswelled using glycerol till they achieve a thickness of 400 microns. In cases of corneal melting, a lower surface irradiation (2.5 mw / cm2) is given to compensate for a thinner cornea. Here, the cross-linking increases the cornea's resistance to digestive enzymes such as collagenase which cause the melting.

Ghanem *et al.*(2010)[44] in a study on 14 individuals with pseudophakic bullous keratopathy (PBK), in Brazil, noted that corneal C3R significantly improved corneal transparency, corneal thickness, and ocular pain, one month post-operatively. However, it did not seem to have a long-lasting effect in decreasing pain and maintaining corneal transparency in patients with PBK.

Recently, C3R has been tried for treating recalcitrant infectious keratitis. Moren *et al.*(2010)[45] in a study on a patient with presumed infectious keratitis in Sweden, observed the positive effects of riboflavin and UV-A collagen cross-linking with a satisfactory final visual outcome. Although these authors felt that this might be a promising new treatment for keratitis, they believed that this treatment should only be considered in

therapy-refractive keratitis or ulceration and not in the first line of defence, since it might have cytotoxic side effects.

Photoactivated riboflavin therapy was performed in seven eyes with refractory infectious keratitis in a study by Panda *et al.*(2013)[46].They observed cessation of corneal melting in all cases following treatment.

Despite the advantages of C3R, fewer complications have been reported in the literature. Wasilewski D *et al.* (2013)[47] in Brazil, reported that corneal cross-linking performed in 36 keratoconus patients induced a considerable decrease in corneal sensitivity; this decrease was more intense at the first week after the procedure, with a progressive recovery up to 6 months.

Bhupesh Bagga *et al.* (2012)[48] reported a case of histopathologically proven endothelial decompensation following C3R, requiring keratoplasty.

Sharma *et al.*(2010) [49] reported a case of pseudomonal keratitis in a 19 year old woman who underwent C3R and this was attributed to the use of bandage contact lens in the early post-op period.

Materials & Methods

MATERIALS AND METHODS

The current investigation was a prospective interventional study on patients with keratoconus who presented to the Cornea Clinic, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamilnadu over a 20 month period (April 2011 to November 2012). This study was approved by the Institutional Ethics Committee.

Fifty eyes were included in this study. The study was done to assess the efficacy of C3R in keratoconus and its role in preventing the progression.

Patients were enrolled in the study if they provided informed written consent to participate, and if they fulfilled the following criteria. (Inclusion criteria)

a) presented with progressive keratoconus for which they had not undergone any other surgical modalities of treatment.
b) keratoconus with an average K value not exceeding 60 D and were less than 25 years of age.
c) had a central corneal thickness of more than 400 microns at the thinnest point; and
d) did not exhibit sub-epithelial and stromal scarring on slit lamp biomicroscopic examination.

Patients were not considered for the study (exclusion criteria) if any one of the following was present:

a) patient was more than 25 years old;

b) central corneal thickness was less than 400 microns at the thinnest point;

c) stationary keratoconus, a scarred cornea or acute hydrops was present;

d) patient was pregnant or lactating;

e) patient suffered from an autoimmune disease or diabetes mellitus; or

f) patient had an active ocular infection.

The main outcome measures evaluated included determination of uncorrected and best corrected visual acuity, spherical equivalent, cylinder value, and corneal topography, before and after C3R, and the frequency, severity and types of adverse effects, if any, following C3R.

Measurements were made at baseline (before the treatment) and at follow-up visits at one month, six months and one year after treatment.

PROCEDURE:

Each patient was informed of the procedure and its possible complications and written informed consent was obtained; the Institutional Ethics Committee approved the study.

The procedure was performed in operating room under sterile conditions. Topical proparacaine hydrochloride (0.5%) eye drops was instilled before the procedure. The central corneal epithelium (9 mm) was then debrided mechanically with a blunt Hockey knife to allow better penetration of riboflavin. This was followed by the application of photosensitizer riboflavin (0.1% solution) to the de-epithelialised cornea every two minutes for a period of approximately 30 minutes. To confirm adequate penetration had occurred, a yellow anterior chamber flare under blue filter was looked for by slit lamp examination.

Exposure to UV-A light was initiated only after ensuring that adequate penetration of riboflavin had occurred, as indicated by the presence of a yellow flare in the anterior chamber. The cornea was then subjected for the next 30 minutes to UV-A radiation (370 nm), in a dose of 3mW/Sq.cm, by an irradiating source placed at a distance of 5 cm from the center of the cornea. To complete photosensitisation and to provide

photoprotection by a barrier effect, riboflavin (0.1% drops) were again instilled every two minutes during the irradiation treatment.

On completion of the procedure, the patient received topical ofloxacin and flurbiprofen eye drops following which a sterile bandage contact lens is applied for three days or till the re-epithelisation of cornea.

Post-procedure, the patient was prescribed topical ofloxacin drops four times a day along with flurbiprofen eye drops four times a day for three days till epithelium healed. Once the epithelium had healed completely, topical prednisolone drops were initiated starting at four times a day and gradually tapering the frequency of application over a month period. Topical lubricating eye drops were also applied four times a day for six months.

Fig. 1 Clinical photo of an eye with keratoconus showing Vogt's striae

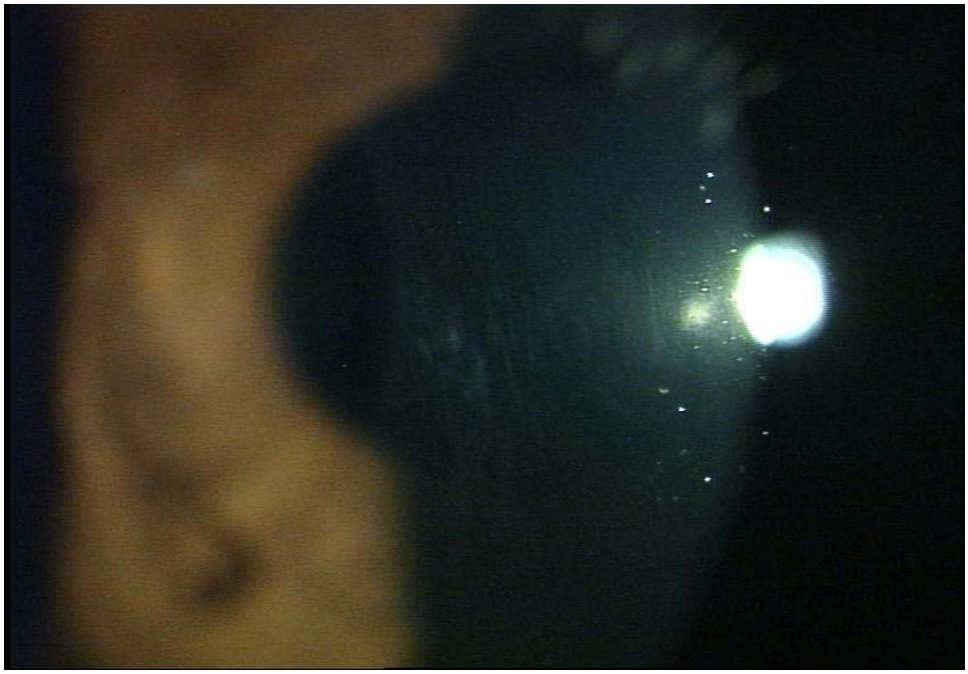


Fig 2 : Clinical photo of an eye with keratoconus showing prominent corneal nerves

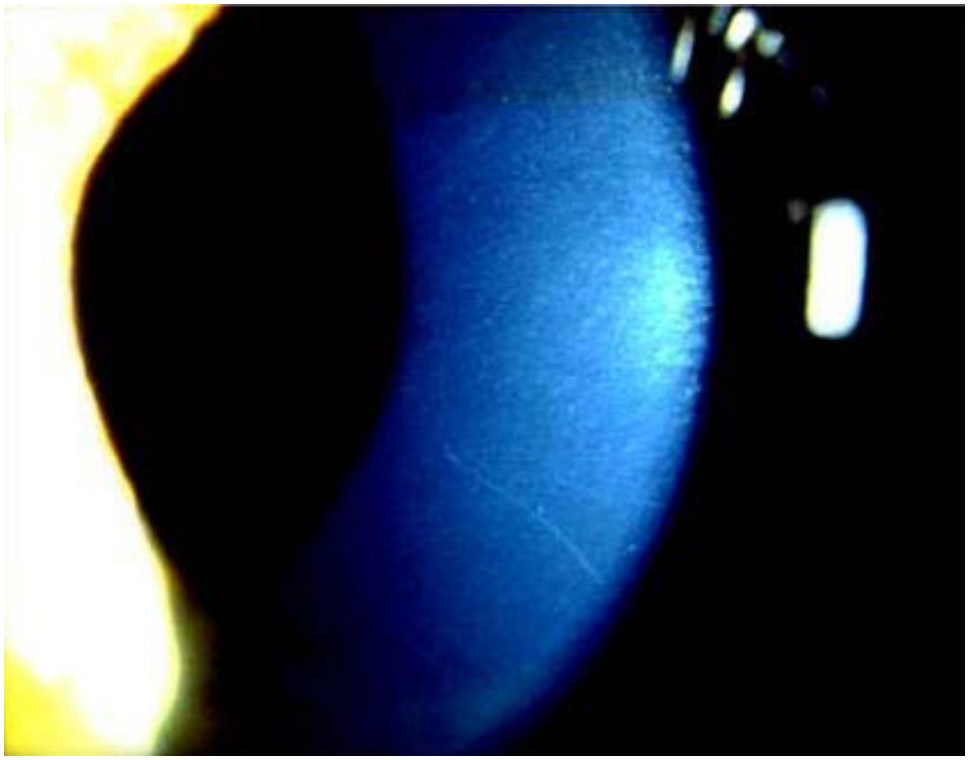


Fig 3: UV-X ™ instrument used for performing C3R

Fig 4: Step1: Measurement of corneal diameter for epithelial debridement

Fig 5: Step 2: Mechanical debridement of corneal epithelium

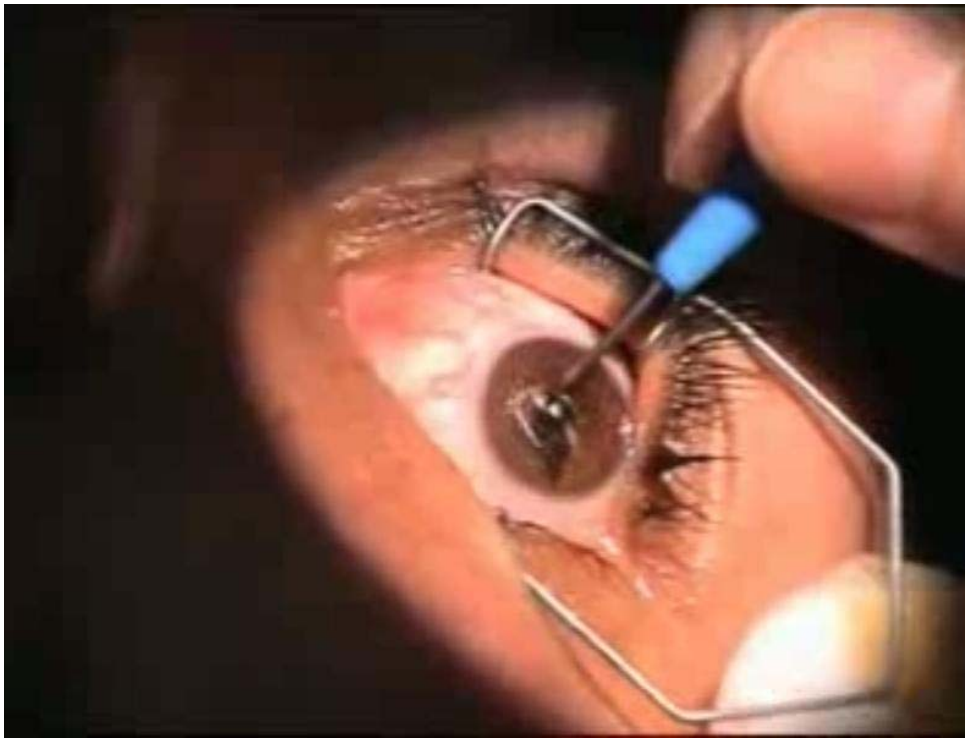
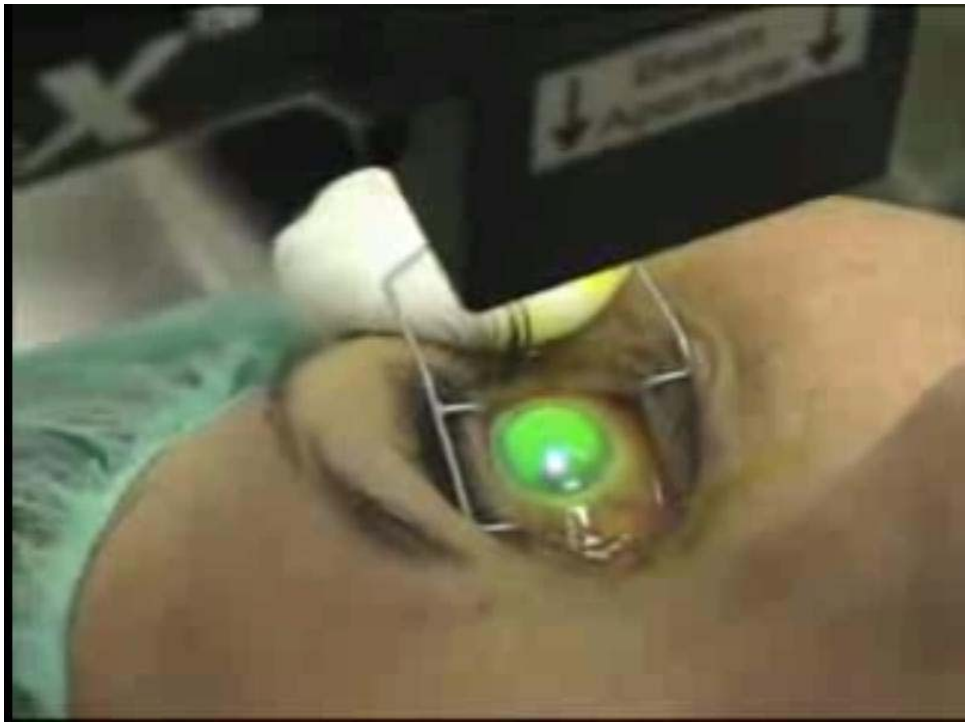


Fig 6: Step 3: Instillation of riboflavin (0.1%) drops every two minutes for 30 minutes

Fig 7: Step 4: Exposure to UV-A (370 nm) with sequential administration of riboflavin (0.1%) drops every two minutes for the next 30 minutes.



Results

RESULTS

The present investigation was a prospective, interventional study on patients who presented with various grades of keratoconus at the Cornea clinic, Joseph Eye hospital, Trichy, over a period of 26 months. (April 2011 to April 2013). Patients were enrolled between April 2011 to November 2012, follow-up was over a period of 6 to 12 months, and data were analysed subsequently.

Sixty individuals with features of keratoconus were seen during the enrolment period. Of these, 22 individuals had at least one exclusion criterion or declined to participate in the study. Finally, 38 patients (50 eyes) who satisfied the inclusion criteria and who consented to undergo corneal collagen cross-linking with riboflavin (C3R) were enrolled in the study.

1) DEMOGRAPHIC ASPECTS

1.1) Gender and Age Distribution:

Fifty eyes of 38 patients were included in this study. There were 20 males (53%) and 18 females (47%). (Chart.1). The mean age of the patients was 19.2 ± 4.5 years (range 11-25 years). There were 12 (31.5%) patients aged between 10-15 years, 11 (28.9%) between 16-20 years and 15 (39.5%) between 21 -25 years. (Chart 2).

Chart 1: Gender distribution of patients with keratoconus undergoing corneal collagen cross-linking at a tertiary eye care hospital

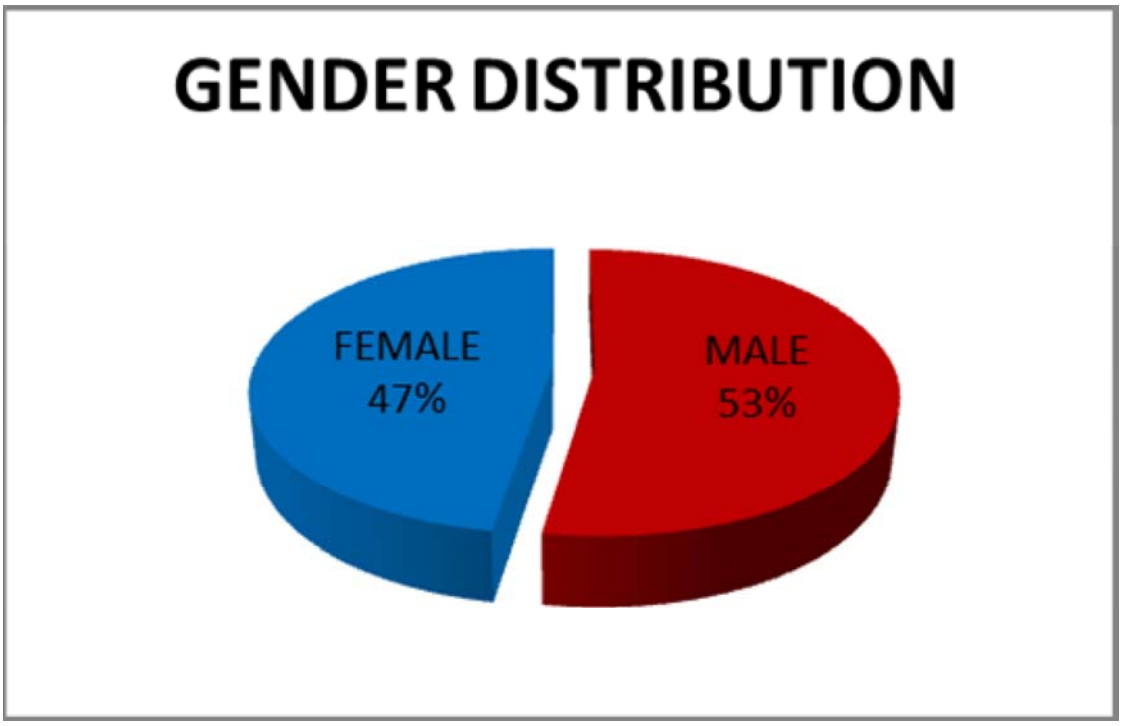
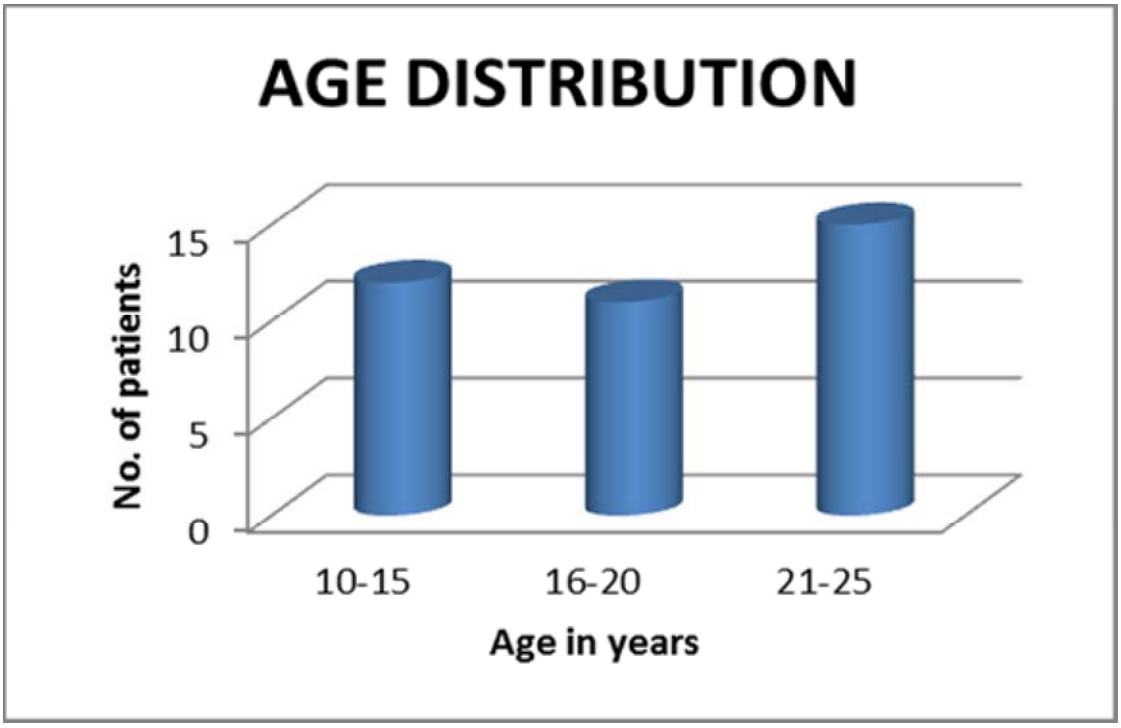


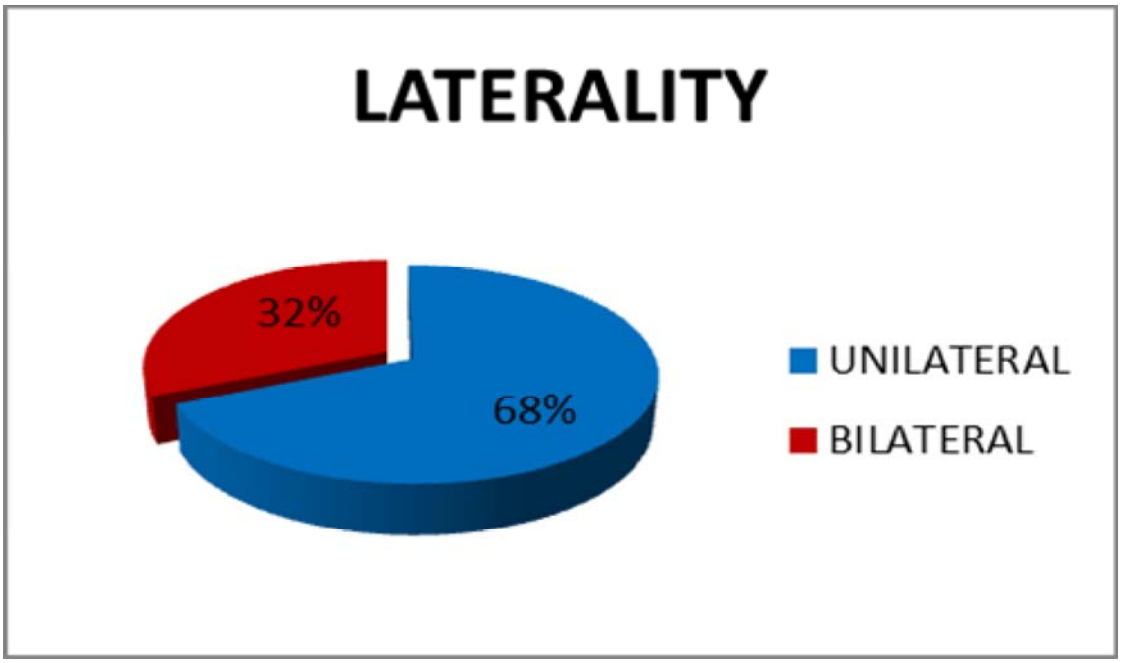
Chart 2: **Age distribution of patients with keratoconus undergoing corneal collagen cross-linking at a tertiary eye care hospital**



1.2) Laterality of the eye undergoing corneal collagen cross-linking with riboflavin:

Twenty six patients (68%) underwent C3R in one eye and 12 patients (32%) in both eyes with the severe eye treated first. (Chart. 3)

Chart 3: Laterality of the eye with keratoconus undergoing corneal collagen cross-linking at a tertiary eye care hospital:



1.3) Indications for corneal collagen cross-linking with riboflavin

Sixteen eyes (32%) had stage I keratoconus, 23 (46%) eyes had stage II keratoconus, and 11 eyes (22%) had stage III keratoconus, based on the Krumeich classification [50].

2) CHANGES IN VISUAL ACUITY:

2.1) Changes in uncorrected visual acuity (UCVA):

The mean UCVA before treatment was 0.18 ± 0.16 (in decimals) (6/60-6/36), and the mean UCVA at the end of one month after C3R was 0.20 ± 0.17(6/36); this improvement was statistically significant (p=0.05) (Table1). The visual acuity continued to improve at 6 months (0.26 ± 0.19 (6/24); p <0.0001) and at one year of follow up (0.32 ± 0.22(6/18), (p <0.0001).Thus there was statistically significant improvement in mean UCVA at all visits post-C3R (Table 1).

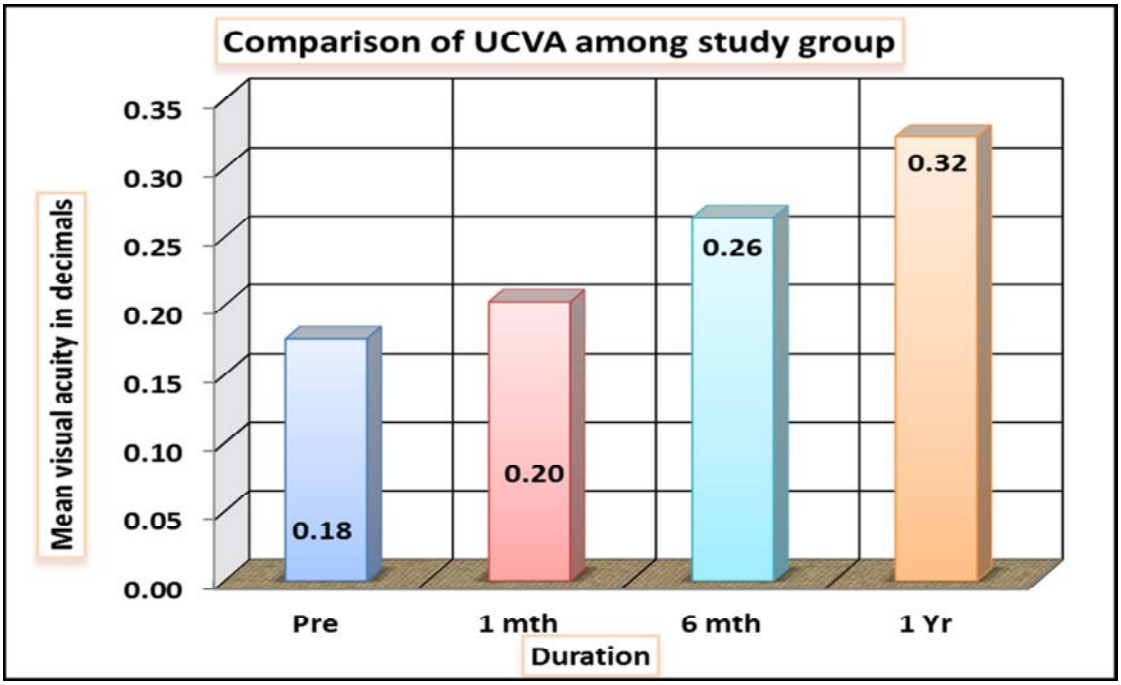
Table 1: Comparison of uncorrected visual acuity in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital:

Examination time	Uncorrected visual acuity Mean ± SD (decimals)	*p-value
Baseline	0.18 ± 0.16	-
1month	0.20 ± 0.17	0.050
6month	0.26 ± 0.19	<0.0001
1year	0.32 ± 0.22	<0.0001

*Note: Paired student 't'-test applied.

SD – Standard deviation.

Chart 4 : Comparison of uncorrected visual acuity (UCVA) among study group



2.2) Changes in best corrected visual acuity (BCVA):

The mean BCVA pre-treatment was 0.51 ± 0.27(6/12), and post-C3R (one month) it was 0.52 ± 0.25(6/12); p= 0.66), this improvement was not of statistical significance (Table 2). However, BCVA showed statistically significant improvement at six months (0.63 ± 0.25(6/12-6/9), (p<0.0001), and at one year (0.76 ± 0.22(6/9-6/6); p <0.0001), post-treatment compared with the pre-treatment data, reaching its maximum at one year with mean BCVA of 0.76 ± 0.22 (in decimals) (6/9-6/6), (p <0.0001) (Table 2).

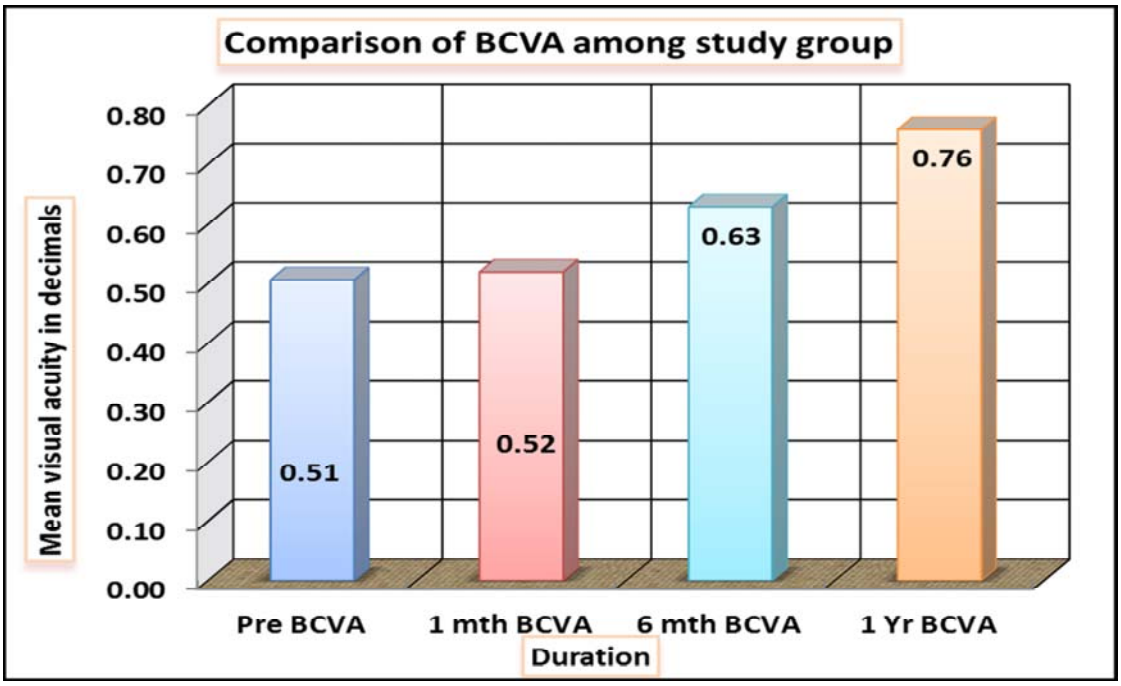
Table 2: Comparison of best corrected visual acuity in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital:

Examination time	Best corrected visual acuity Mean ± SD (decimals)	*p-value
Baseline	0.51 ± 0.27	-
1month	0.52 ± 0.25	0.664
6month	0.63 ± 0.25	<0.0001
1year	0.76 ± 0.22	<0.0001

*Note: Paired student 't'-test applied.

SD – Standard deviation.

Chart 5 : Comparison of BCVA among study group



3) CHANGES IN SPHERICAL EQUIVALENT AND CYLINDER VALUE :

3.1) Changes in the Spherical equivalent (SE)

The baseline mean SE was -4.60 D (SD ± 2.99), (Table 3). The mean SE was found to have reduced to -4.45 D (SD ± 2.85) at one month following C3R; however this reduction was not statistically significant (p = 0.35). Further reduction in SE value compared to baseline mean SE was noted at the end of 6 months (-3.97 D (SD ± 2.60); p = 0.001); and at one year (-3.63 D (SD ± 2.70); p = 0.001) (Table 3). Thus the mean SE decreased during the follow up period, with the difference from the baseline reaching statistical significance at 6 months and one year. (p = 0.001) (Table 3).

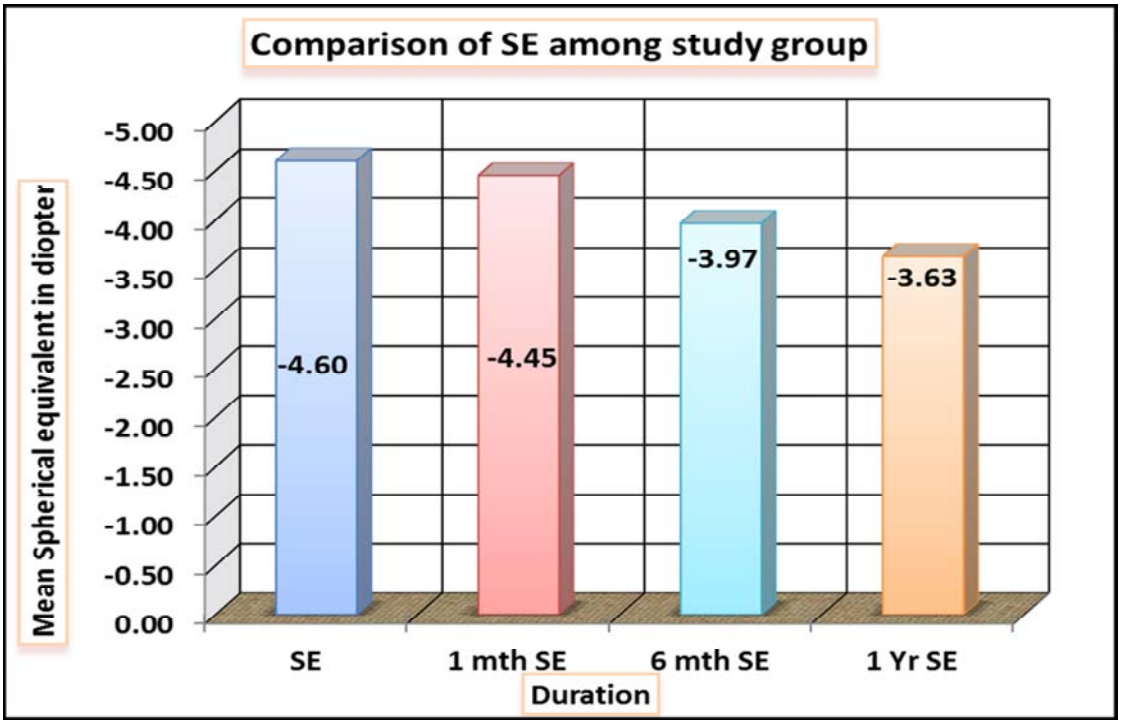
Table 3: Comparison of spherical equivalent in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital:

Examination time	Spherical equivalent Mean ± SD (diopters)	*p-value
Baseline	-4.60 ± 2.99	-
1month	-4.45 ± 2.85	0.358
6month	-3.97 ± 2.60	0.001
1year	-3.63 ± 2.70	0.001

*Note: Paired student ‘t’- test applied.

SD – Standard deviation

Chart 6 : Comparison of Spherical equivalent (SE) among study group



3.2) Changes in the Cylinder value:

The mean cylinder value at baseline was -3.70 D (SD ± 1.86), and reduction in the cylinder value throughout the one year follow-up period was observed post-treatment (Table 4). The mean cylinder value at the end of one month was -3.18 D (SD ± 1.4); p=0.01), at 6 months was -2.71D (SD ± 1.52); p<0.0001); and at one year was -2.39 D (SD ± 1.40), (p<0.0001) (Table 4).

This reduction in mean cylinder value from baseline throughout the follow-up at one month, 6 months and at one year post- C3R was found to be statistically significant at all post-operative visits (p<0.0001) (Table 4).

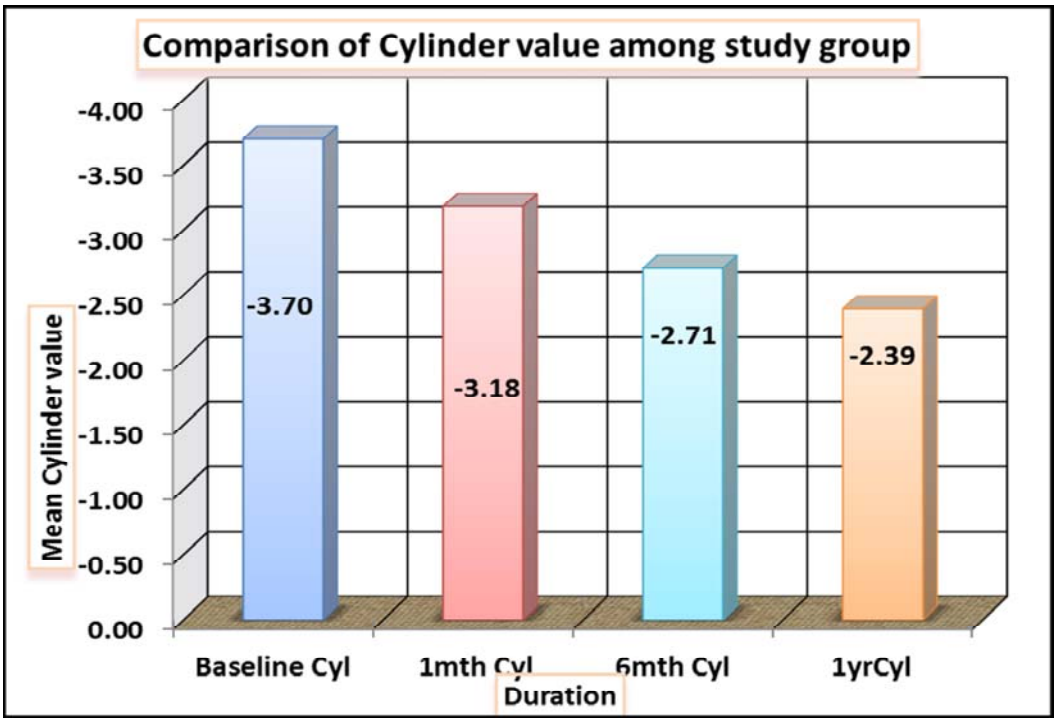
Table 4: Comparison of cylinder value in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital:

Examination time	Cylinder value Mean ± SD (diopters)	*p-value
Baseline	-3.70 ± 1.86	-
1month	-3.18 ± 1.4	0.013
6month	-2.71 ± 1.52	<0.0001
1year	-2.39 ± 1.40	<0.0001

*Note: Paired student 't'-test applied.

SD – Standard deviation.

Chart 7: Comparison of cylinder value among study group



4) CHANGES IN KERATOMETRY READINGS:

4.1) Changes in keratometry value of steeper meridian (Kmax):

The mean keratometry value of the steeper meridian (Kmax) at baseline was 53.49 D (SD ± 4.35), (range: 47 - 60.25 D) (Table 5). The mean Kmax at one month post-treatment was found to have reduced to 52.37 D (SD ± 8.68; p=0.001).There was further reduction at 6 months (51.94 D (SD ± 8.75), p <0.0001); and at one year (51.67 D (SD ± 8.76); (p <0.0001) (Table 9). This reduction in the Kmax value from baseline was statistically significant at all post-operative visits (p <0.0001).

The quantum of reduction in the mean K max value compared to the baseline was 0.14 D at one month, 0.63 D at six months and was 0.90 D at one year (Table 5).

Table 5: Comparison of Kmax in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital

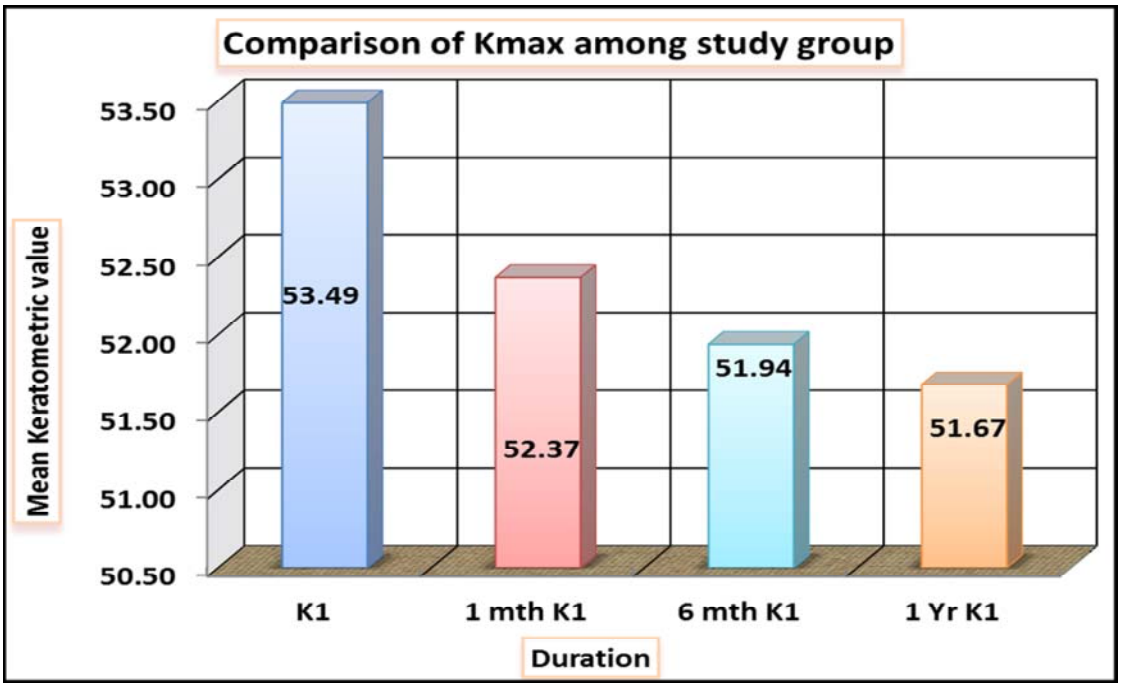
Examination time	Kmax Mean ± SD (diopters)	*p-value
Baseline	53.49 ± 4.35	-
1month	52.37 ± 8.68	0.001
6month	51.94 ± 8.75	<0.0001
1year	51.67 ± 8.76	<0.0001

*Note: Paired student ‘t’-test applied.

SD – Standard deviation.

Kmax - keratometry value of the steeper meridian.

Chart 8 : Comparison of Kmax among study group



4.2) Changes in keratometry value of the flatter meridian (Kmin):

The mean keratometry value of the flatter meridian (Kmin) was 47.41 D (SD ± 3.50) (range: 45.12 - 56.12 D) prior to treatment (Table 6). At one month post-C3R, the Kmin value was 46.41 D (SD ± 7.53); p=0.001); and at 6 months post-C3R was 46.09 D (SD ± 7.53); p <0.0001); and at one year post-C3R, it was 45.91 D (SD ± 7.53); p <0.0001). This constant reduction in Kmin value from baseline throughout the follow-up period was statistically significant. (p <0.0001).

The quantum of reduction of the mean Kmin value was found to be 0.11 D at one month, 0.44 D at 6months , and 0.63 D at one year follow-up, respectively (Table 6).

Table 6: Comparison of Kmin in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital:

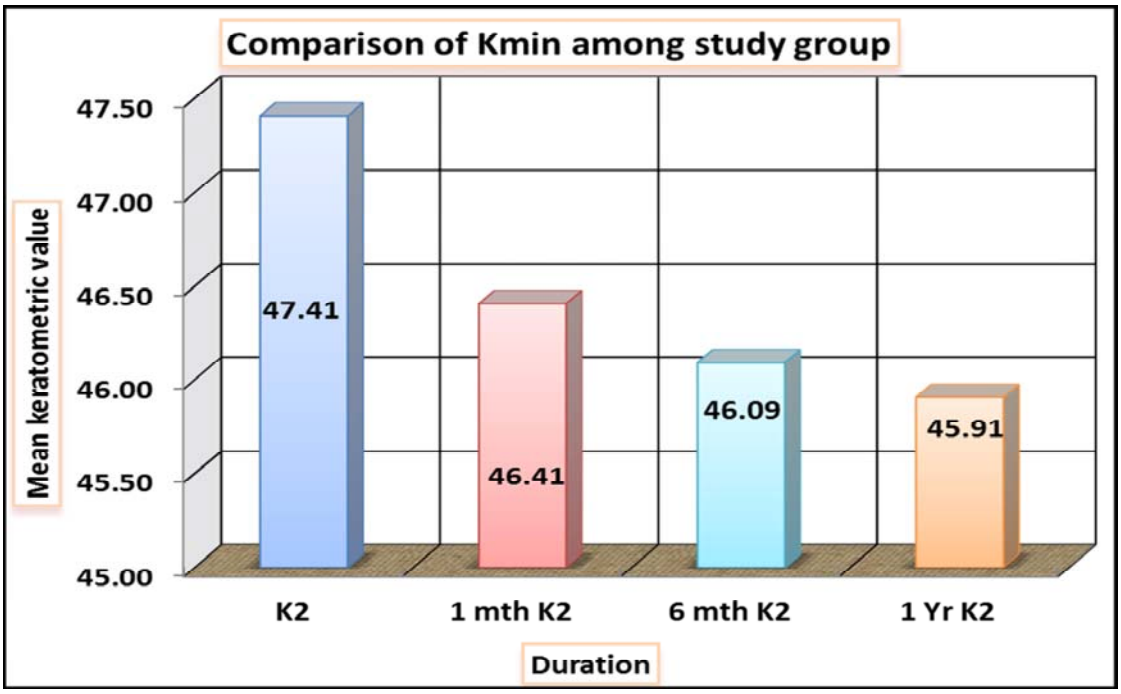
Examination time	Kmin Mean ± SD (diopters)	*p-value
Baseline	47.41 ± 3.50	-
1month	46.41 ± 7.53	0.001
6month	46.09 ± 7.53	<0.0001
1year	45.91 ± 7.53	<0.0001

*Note: Paired student 't'-test applied.

SD – Standard deviation.

Kmin- keratometry value of the flatter meridian.

Chart 9: Comparison of Kmin among study group



4.3) Changes in average keratometry value (Kavg):

The mean average keratometry value (Kavg) at baseline was 50.45D (SD ± 3.75) (range: 47.25-57.62 D) (Table 7).There was reduction in Kavg value at one month post-C3R to 49.39 D (SD ± 8.03), and this reduction was statistically significant (p =0.002). On follow-up, the Kavg value was found to be further reduced to 49.01 D (SD ± 8.06), p <0.0001) at 6 months, and to 48.79 D (SD ± 8.06), p<0.0001) at one year post-C3R (Table 7). This reduction in Kavg value on follow-up was of statistical significance.

This study also recorded a reduction in the mean Kavg value during the follow-up, by a mean of 0.12 D at one month, 0.54 D at 6 months, and 0.77 D at one year post-C3R (Table 7).

Table 7: Comparison of K avg in eyes with keratoconus undergoing corneal collagen cross-linking with riboflavin at a tertiary eye care hospital

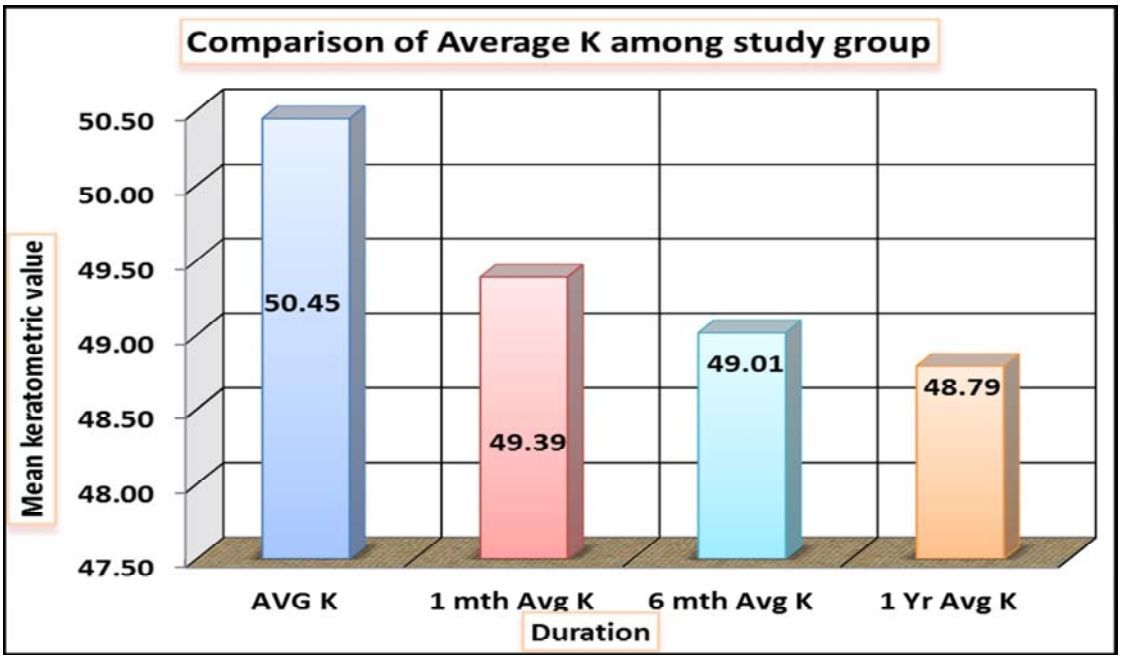
Examination time	Kavg Mean ± SD (diopters)	*p-value
Baseline	50.45 ± 3.75	-
1month	49.39 ± 8.03	0.002
6month	49.01 ± 8.06	<0.0001
1year	48.79 ± 8.06	<0.0001

*Note: Normality test failed, thus Wilcoxon Signed ranks test applied.

SD – Standard deviation.

Kavg - mean average keratometry value.

Chart 10 : Comparison of Kavg among study group



5) Patients and ocular factors affecting the response to treatment:

In this study, eyes that showed a reduction in Kavg value by more than 0.5 D from the baseline, at the end of one year follow-up were considered improved and those eyes which showed reduction of less than 0.5 D were considered stable.

Thirty-four eyes showed improvement and 10 eyes remained stable at the end of one year follow-up in this study.

5.1) Gender

In the improved group (n=34 eyes), there were 13 males and 13 females (34 eyes of 26 patients) and in the stable group (n=10 eyes), there were five males and four females (10 eyes of 9 patients); this difference was not statistically significant. (χ^2=0.085; (d. F=1); p=0.77).

5.2) Age

In the improved group (n=34 eyes), 11 patients were aged below 20 years and 15 patients were aged above 20 years; and in the stable group (n=10 eyes), four patients were aged below 20 years and five patients were aged above 20 years. There was no statistically significant difference with respect to age affecting the response to treatment.

(χ^2=0.015; (d. F=1); p=0.90).

5.3) Central corneal thickness

There were 25 eyes (73.52%), with central corneal thickness more than 450 microns and nine eyes (26.48%) with central corneal thickness less than 450 microns in the improved group (n=34 eyes). In the stable group (n=10 eyes), there were eight eyes (80%) with central corneal thickness more than 450 microns and two eyes (20%) with central corneal thickness less than 450 microns; this was of no statistical significance in affecting the treatment outcome. (χ^2=0.159; (d. F=1); p=0.69).

5.4) Average keratometry reading

The baseline mean K avg value was more than 50 D in 15 eyes (44.12%) and less than 50 D in 19 eyes (55.88%) in the improved group (n=34 eyes), and the baseline mean K avg value was more than 50 D in seven eyes (70%) and less than 50 D in three eyes (30%) in the stable group (n=10 eyes). There was no statistically significant difference in the pre-treatment Kavg value influencing the response to treatment.
(χ^2=2.072; (d. F=1); p=0.15).

In this study, an increase in Kavg value compared to baseline value by less than 0.5 D was observed in four eyes (8%), at the end of one year of follow-up. Further analysis was not done because of smaller size of the group.

Table 8: Patients and ocular factors affecting the response to treatment

Parameters		No. of improved eyes (%)	P value (Chi-square test)
Gender	(Male)	13 (72.22%)	0.77
	(Female)	13 (76.47%)	
Age	(< 20 years)	11 (73.33%)	0.90
	(> 20 years)	15 (75%)	
CCT	(> 450 microns)	25 (75.75%)	0.69
	(< 450 microns)	9 (81.81%)	
Avg K	(< 50D)	19 (86.36%)	0.15
	(> 50D)	15 (68.18%)	

6) Complications

Two patients in this study developed complications in the form of corneal ulcer within the first month of the procedure. Both the patients were started on topical broad spectrum antibiotics. On further follow-up, one patient improved, but corneal scarring occurred with resultant steepening of average K values. On the other hand, in the other patient, the ulceration progressed necessitating therapeutic penetrating keratoplasty. Thus complications were noted in only two (4%) of 50 eyes in this study.

Fig. 8: Corneal topography map of a study eye showing typical asymmetric bow-tie pattern of keratoconus prior to C3R

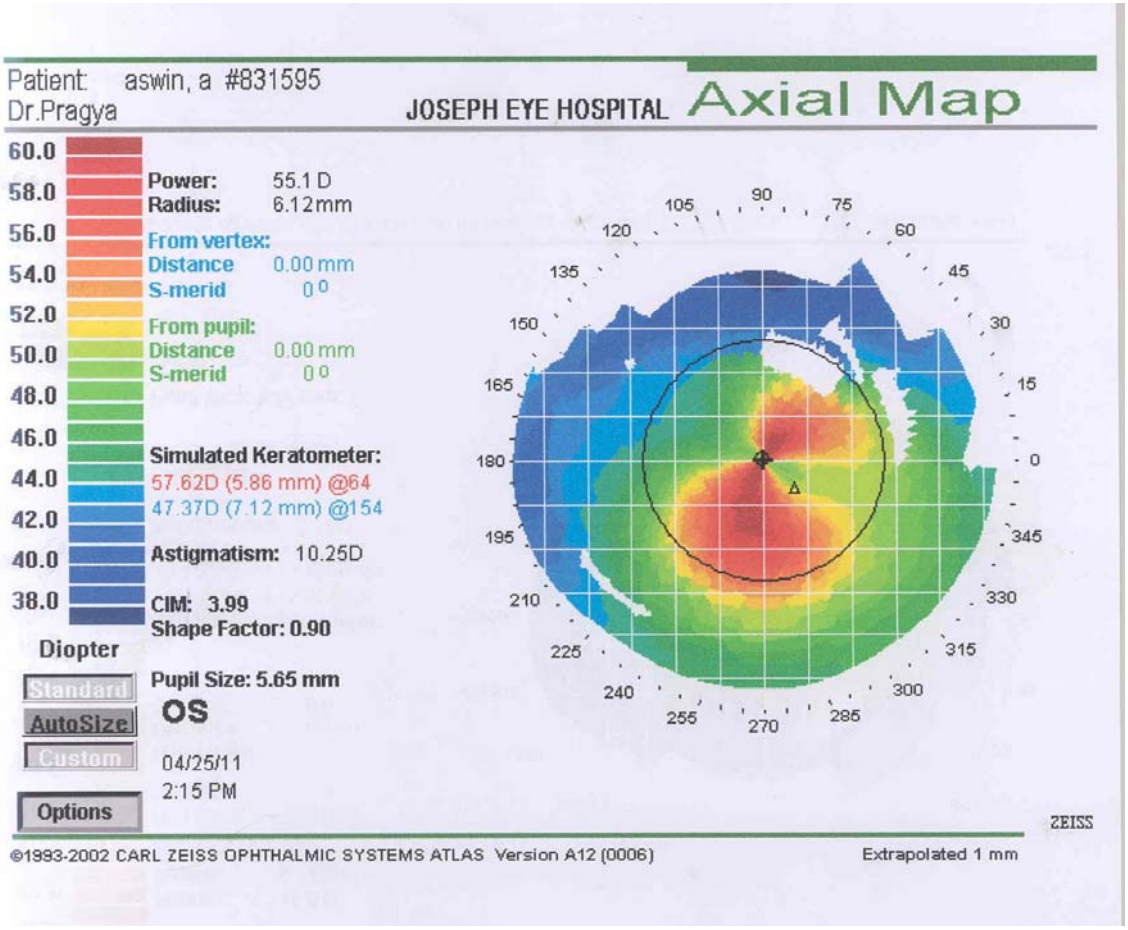
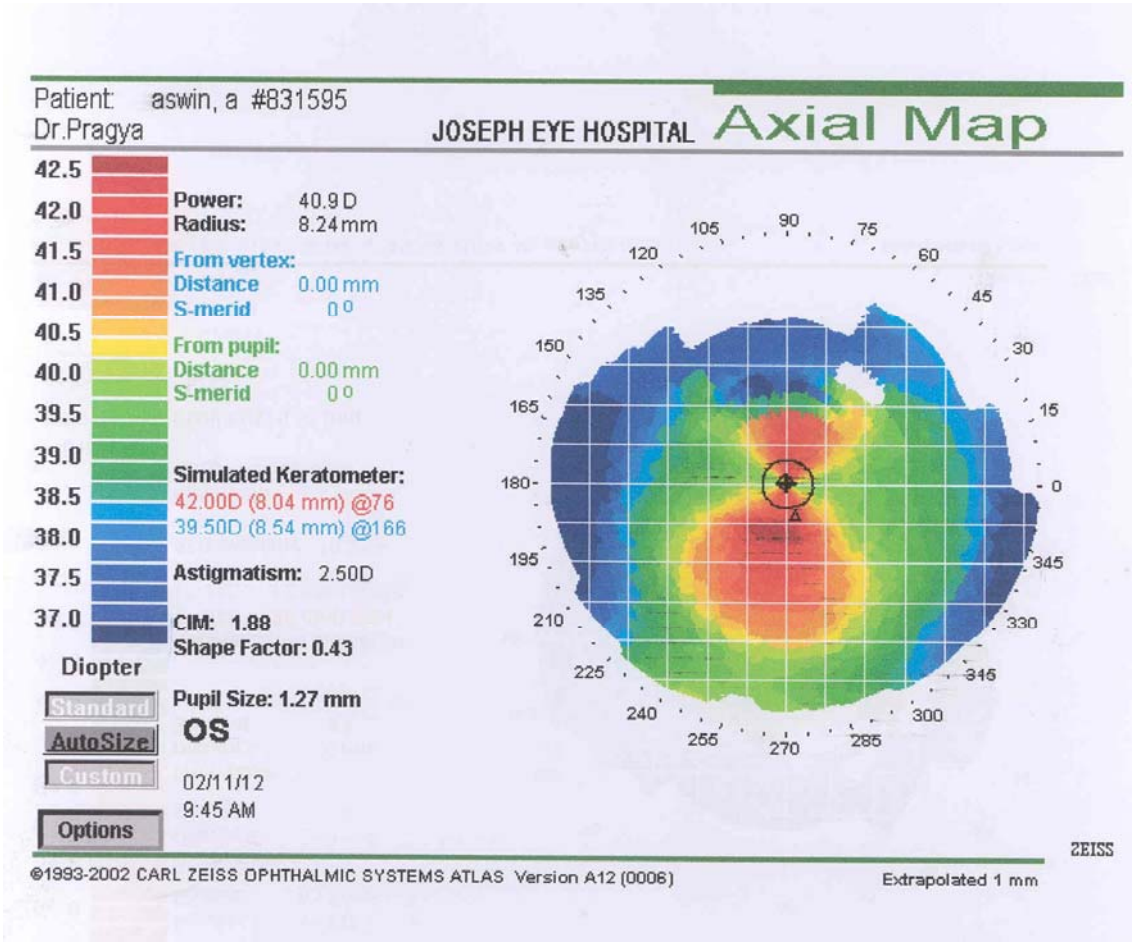


Fig. 9:Corneal topography map of the same study eye showing an improvement in the keratometric readings post-C3R



Discussion

DISCUSSION

Corneal collagen cross-linking with riboflavin (C3R) is a promising new treatment modality for keratoconus patients as it provides various advantages over the conventional treatment modalities for corneal ectasia. The specific advantage of C3R over the other modalities is that it halts the progression of the disease and, in some cases, it also causes a partial reversal of the pre-existing ectasia, and also defers the need for keratoplasty.

The basis underlying this technique was understood only after a detailed study of the pathogenic mechanisms of ectatic disorders. C3R increases the mechanical and chemical stability of the corneal tissue by creating additional chemical covalent bonds in the corneal stroma by means of highly localized photopolymerization [(4)] ; this leads to either slowing down or total arrest of the progression of ectasia [(2,4)]. According to one study, C3R increases corneal rigidity by 328.9% [(5)]. In addition to keratoconus, C3R is now being sucessfully tried for the treatment of pellucid marginal degeneration, progressive post-LASIK keratectasia (PPLK), bullous keratopathy, infectious keratitis, and corneal melts.

In the present study, an attempt was made to study the efficacy of C3R in patients with progressive keratoconus and its outcome was followed-up over a period of one year. The indicators which were used to assess the outcome of C3R were UCVA, BCVA, SE, cylindrical error, keratometry values (Kmax, Kmin, Kavg), and the rate of development of complications if any.

Much research has been done on this topic in the western population where the procedure showed encouraging results. However, with respect to the Asian population, only a few studies have been done. Hence, the current study is of great relevance.

Demographics:

In the current study, 38 patients were included of which 50 eyes underwent C3R. All the patients were followed up for a minimum period of one year. Unlike a few other studies, such as by Raiskup *et al.* (2008)[32] who studied 480 eyes of 272 patients, the sample size included in this study was relatively small.

The ratio of male: female patients in the current study was 1:1 (Chart 1). A study by Caporossi *et al.* (2011)[51] had a male: female ratio of 4:1. In other reported literature [(1)], the male: female ratio was 2:1. This

possibly reflects the variation in the incidence of the disease in the Asian population, as compared to its Western counterparts.

Keratoconus, being a bilateral, asymmetric disease, presents with one eye affected more than the other in most circumstances. This is seen in the current study as 68% of the patients included in the study had a unilateral disease requiring treatment at presentation (Chart 3).

The mean age of patients included in this study was 19.2 ± 4.5 years (range 11-25 years) (Chart 2). The other related studies done in the west show a higher mean age of patients. In the trial done by Wollensak *et al.* (2003)[2] and the one done by Caporossi et al .(2006)[8], mean age groups of 31.7 years and 31.4 years, respectively, were reported.

In the study by Caporossi *et al.*(2011)[51], the patients were divided into 3 groups on basis of age. The study concluded that the benefit of the C3R procedure was more in the paediatric group and in patients below 26 years of age. Koller *et al.*(2009)[52] observed a poor outcome of the procedure when performed in individuals above 35 years of age.

A previous study done on the Indian population by Agrawal *et al.*(2009)[36] included patients with a mean age of 16.9 ± 3.5 years (range :

12-39 years). This might point to the fact that the incidence of keratoconus may be higher in the younger age groups in the Asian population.

The speed of keratoconus progression in paediatric age group as compared to adult keratoconus is higher as proven in many studies. Chatzis and Hafezi *et al.* (2012)[53] found that 88% of keratoconic patients in the paediatric age group showed a progression from their initial evaluation. Soeters *et al.*, (2011)[54] noted progression of keratoconus to be rapid in paediatric patients, which ranged from 2.6 D in seven weeks to 5.0 D over a period of one year. These observation suggest that treatment at presentation of disease may be initiated as opposed to waiting for signs of progression.

However, the present study did not observe any impact of the age parameter on visual and topographic outcome after C3R (Table 8). Thus the importance of doing C3R during the early course of disease is highlighted especially in the Asian population in whom the age of presentation of keratoconus was found to be younger. This might delay or eliminate the need to perform more invasive surgeries such as lamellar or penetrating keratoplasty.

In the current study, the patients included were staged by the Krumeich classification [(50)] with the maximum percentage falling in

stage II keratoconus. This study did not attempt to infer any positive or negative impact on the outcome parameters based on these criteria. However, in a study by Koller *et al.*(2009)[52], an observation was made that a poor post-operative prognosis was seen if C3R was done in patients having a high keratometry value of more than 58 D (stage III).

In the current study, pre-operatively, the central corneal thickness was measured by a pachymetry for all the patients enrolled in the study. This parameter was not measured on subsequent follow-up visits. The current study did not observe any influence of pre-operative corneal thickness in the functional outcome of the procedure (Table 8).

In the landmark Sienna CXL trial conducted by Caporossi *et al.* (2012)[38], the patients were divided into two groups on the basis of CCT (< 450 and > 450 microns). It was observed that, the visual outcome in the form of UCVA and BCVA and also the visual recovery were better in the group with thicker cornea. In a study conducted by Kankaria *et al.*, (2013)[55], it was observed that the CCT reduced in value at one month and this gradually recovered in subsequent follow up visits. In the same study, the authors suggested that the trans-epithelial variety of C3R may be tried in patients with thin corneas. Greenstein *et al.* (2013)[56,] however did not

find any impact of pre-operative corneal thickness on the outcome of the procedure, as observed in the current study.

Uncorrected visual acuity

In the current study, an observation was made that the mean UCVA (in decimals) calculated at all post-operative follow-up visits showed a statistically significant improvement in comparison to the pre-operative mean UCVA, improving from 0.18 to 0.32 at the final follow-up visit at one year (Table 1). Similar studies conducted in the west, such as one done by Caporossi *et al.* (2010)[31] demonstrated a statistically significant improvement in terms of Snellen lines at all follow-up visits upto two years after the procedure. In the Sienna CXL trial conducted on paediatric patients, Caporossi *et al.*(2012)[38] observed a statistically significant improvement in the mean UCVA.

Studies conducted on the Indian population showed comparable results which were encouraging. Arora *et al.* (2012)[57] demonsrated an improvement in UCVA at the end of one year. Thus there was a statistically significant improvement post the C3R procedure across all the studies.

Best corrected visual acuity

Concurrent to the improvement in the UCVA, the BCVA also showed a gradual improvement in comparison to the baseline BCVA in the present study (Table 2). However the improvement in comparison to the baseline BCVA was statistically significant only at 6 months and one year follow-up visits. The one month value was not found to be statistically significantly different from the pre-operative value. The mean BCVA improved from 0.51 to 0.76 at one year (Table 2).

A study by Caporossi *et al*. (2010)[31] demonstrated a mean improvement in BCVA at all the follow-up visits until two years from the procedure. Raiskup *et al.* (2008)[32] in their long term study showed an improvement in BCVA in terms of Log MAR value at the end of 6 years. In other one-year follow up studies by Witting Silva *et al.* (2008)[58] and Hersh *et al.* (2011)[59] also showed a Log MAR improvement similar to that seen in the present study. Caporossi *et al*. in the Sienna CXL paediatric trial (2012)[38] observed a statistically significant improvement at final follow-up visit of 36 months.

Studies conducted in the Indian population had comparable results, Arora *et al.* (2012)[57] found an improvement in mean BCVA at the end of

one year. In another study by Agrawal *et al.* (2009)[36], an improvement in terms of Snellen lines was observed at the end of one year.

Thus, the procedure shows a statistically significant improvement in the BCVA, irrespective of the population demographic. Also, the lack of statistically significant improvement in BCVA in the current study at one month follow-up might suggest the late stabilization of the visual and topographic parameters and might be indicative of the post-operative visual recovery and rehabilitation time, or might reflect the small sample size studied.

Spherical equivalent

Spherical equivalent was calculated as the sum of the spherical value (DS) and half of the cylindrical value (1/2DC).

In the current study, the mean pre-operative SE value was found to show a gradual reduction at all the post-operative visits (Table 3). This reduction in the SE when compared to the pre-operative values was found to be statistically significant.

Agrawal *et al.* (2009)[36] in a study conducted in the Indian population, also observed a similar reduction in the values of both the sphere and the SE. Another study by Vinciguerra *et al.* (2012)[60] observed a

reduction in the spherical equivalent value, two years after the CXL procedure.

Thus, it appears that reduction of the value of spherical equivalent post-C3R occurs in both the western and Asian patients.

Cylinder value

The current study also compared the mean reduction of the cylindrical value in the group (Table 4). In comparison to the preoperative mean cylindrical value of -3.70 D, statistically significantly lower values were noted at all follow-up visits, and a value of -2.39 D being noted at the final post-operative visit at one year.

Studies by Caporossi *et al.* (2006)[8] and Saffarian L *et al.* (2010)[61] demonstrated a similar reduction in cylindrical value which might be indicative of gradual corneal flattening post-C3R.

Keratometric readings

The topographic indices, such as the Kmax (the steeper meridian), Kmin (the flatter meridian) and the Kavg (average keratometry) values over each of the follow-up visits at one month, 6 months and one year were observed in the present study (Table 5, 6, 7).

In this study, Kmax showed a gradual flattening in comparison with the pre-operative values at all the post-operative visits in a statistically significant manner. Similarly the Kmin also showed a statistically significant reduction from the pre-operative values at all post-operative visits. The Kavg values also showed a similar trend (Table 7). The reduction in Kavg was also found to be statistically significant.

In other studies conducted on western population, a similar trend was observed. Witting Silva *et al.* (2008)[58] and Hersh *et al.* (2011)[59] observed a reduction in the value of Kmax at the end of their one-year study. Greenstein *et al.* (2013)[56] observed a flattening of the steeper meridian (Kmax) in patients with severe disease having K value more than 55 D.

A study conducted by Agrawal *et al.* (2009)[36] in the Indian population also observed similar results at the end of one year. These results were comparable to that of the present study. Caporossi *et al.* (2010)[31] studied the topographic findings and observed the reduction in the mean average K in a statistically significant manner over a period of two years. This reduction was gradual and statistically significant.

Another long term, large sample study by Raiskup *et al.* (2008)[32] demonstrated similar results. Also, Kankaria *et al.* (2013)[55] noticed a

transient worsening in the topographic indices in the early post-operative period. These indices stabilized on subsequent visits without any intervention.

Thus, most studies in the literature cited showed a gradual reduction in the keratometric values after C3R. All the visual and topographic indices observed over the period of one year after the procedure, showed a trend of either halting or partially reversing the disease process. The improvement was seen irrespective of the demographical factors, such as age, gender and other pre-operative indices such as corneal thickness and the average keratometry value (Table 8).

In the current study, the eyes which showed an improvement of more than 0.50 D were considered to be statistically significant and the ones in which the improvement was less than 0.50 D were considered to be stable. Out of the 50 eyes in this study, 68% (34 eyes) showed improvement, whereas 20% (10eyes) remained stable. However an increase in the average K by less than 0.50 D was noted in four eyes. Two eyes that developed ulcerative keratitis were excluded in the analysis. This indicated that C3R gives a beneficial response to arrest the progression of keratoconus overall.

Complications

A complication rate of 4% (2 eyes) was observed in this study. The development of keratitis in both eyes was attributed to the usage of bandage contact lens, post-procedure. Both patients were started on topical broad spectrum antibiotics. One of the patients showed improvement whereas the other patient who did not respond to topical therapy, required penetrating keratoplasty. On culture report, the organism was found to be Staphylococcus aureus.

Sharma N *et al.* (2010)[48] observed a case of pseudomonas keratitis after C3R procedure. This was an isolated case report. Gautham *et al.* (2013)[62] reported a case of infectious keratitis due to Microsporidium after collagen crosslinking (CXL). A few other complications were reported in studies by Bakshi *et al.* (2012)[63]. They had a patient who developed corneal haze following C3R. This resolved on treatment with topical steroid therapy.

Similarly in the Sienna CXL trial by Caporossi *et al.* (2010)[31], 9.8% patients were found to have moderate corneal haze which resolved without treatment; this haze was not found to be visually significant.

Koller *et al.*(2009)[52] demonstrated an increased risk of complications and increased failure rates in patients of age more than 35 years and Kmax value more than 58 D undergoing C3R. This indicates that the procedure should not be delayed for a long period of time and ideally should be performed at the time of presentation for the best results. Other studies like the one by Chatzis and Hafezi at al., (2012)[51] did not observe any visually significant complication.

Summary

SUMMARY

Corneal collagen cross-linking with riboflavin is a simple, safe and an effective technique which is fast emerging as a treatment modality for corneal ectasia. The aim of this study was to evaluate the safety and efficacy of corneal collagen cross-linking in keratoconus and its role in preventing the disease progression.

The outcome measures evaluated were uncorrected visual acuity, best corrected visual acuity, spherical equivalent, cylindrical error, keratometry values (Kmax, Kmin, Kavg), and the rate of development of complications, if any.

The current investigation was a prospective interventional study on patients with keratoconus who presented to the Cornea Clinic, Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, Tamilnadu over a 20 month period (April 2011 to November 2012).

Fifty eyes of 38 patients, comprising 20 males (53%) and 18 females (47%) with a mean age of 19.2 ± 4.5 years (range: 11-25 years), who presented with progressive keratoconus during the study period, who satisfied the inclusion criteria and who provided consent for undergoing C3R were enrolled in the study.

Among them, 16 eyes (32%) had stage I keratoconus, 23 (46%) eyes had stage II keratoconus, and 11 eyes (22%) had stage III keratoconus, based on the Krumeich classification [50].

Twenty six patients (68%) underwent C3R in one eye and 12 patients (32%) in both eyes with the severe eye treated first.

The mean UCVA before treatment was 0.18 ± 0.16 (in decimals) (6/60-6/36), and the mean UCVA at the end of one month after C3R was 0.20 ± 0.17(6/36); this improvement was statistically significant (p=0.05) (Table1). The visual acuity continued to improve at 6 months (0.26 ± 0.19 (6/24); p <0.0001) and at one year of follow up (0.32 ± 0.22(6/18); (p <0.0001).Thus, there was statistically significant improvement in mean UCVA at all visits post-C3R (Table 1).

The mean BCVA pre-treatment was 0.51 ± 0.27(6/12), and post-C3R (one month) it was 0.52 ± 0.25(6/12); p=0.66), this improvement was not of statistical significance (Table 2).However, BCVA showed statistically significant improvement at six months (0.63 ± 0.25(6/12-6/9); (p <0.0001), and at one year (0.76 ± 0.22(6/9-6/6); p <0.0001), post-treatment compared with the pre-treatment data, reaching its maximum at one year with mean BCVA of 0.76 ± 0.22 (in decimals) (6/9-6/6), (p <0.0001) (Table 2).

The baseline mean SE was -4.60 D (SD ± 2.99), (Table 3). The mean SE was found to have reduced to -4.45 D (SD ± 2.85) at one month following C3R; however this reduction was not statistically significant (p =0.35). Further reduction in SE value compared to baseline mean SE was noted at the end of 6 months (-3.97 D (SD ± 2.60); p =0.001); and at one year (-3.63 D (SD ± 2.70); p=0.001) (Table 3). Thus the mean SE decreased during the follow-up period, with the difference from the baseline reaching statistical significance at 6 months and one year. (p =0.001) (Table 3).

The mean cylinder value at baseline was -3.70 D (SD ± 1.86), and reduction in the cylinder value throughout the one year follow- up period was observed post treatment (Table 4). The mean cylinder value at the end of one month was -3.18 D (SD ± 1.4); p=0.01), at 6 months was -2.71D (SD ± 1.52); p<0.0001); and at one year was -2.39 D (SD ± 1.40), (p<0.0001) (Table 4).

This reduction in mean cylinder value from baseline throughout the follow-up at one month, 6 months and at one year post-C3R was found to be statistically significant at all post-operative visits (p<0.0001) (Table 4).

The mean keratometry value of the steeper meridian (Kmax) at baseline was 53.49 D (SD ± 4.35), (range: 47 - 60.25 D).The mean

keratometry value of the flatter meridian(Kmin) was 47.41 D (SD ± 3.50) (range: 45.12 - 56.12 D).The mean average keratometry value (Kavg) was 50.45 D (SD ± 3.75) (range: 47.25-57.62 D).There was statistically significant improvement in these parameters in comparison to the baseline during the follow-up period.

The mean keratometry value of the steeper meridian (Kmax) at baseline was 53.49 D (SD ± 4.35), (range: 47 - 60.25 D) (Table 5). The mean Kmax at one month post-treatment was found to have reduced to 52.37 D (SD ± 8.68; p=0.001).There was further reduction at 6 months (51.94 D (SD ± 8.75), p <0.0001); and at one year (51.67 D (SD ± 8.76); (p <0.0001) (Table 5).

This reduction in the Kmax value from baseline was statistically significant at all post-operative visits (p <0.0001).

The quantum of reduction in the mean K max value compared to the baseline was 0.14 D at one month, 0.63 D at six months and was 0.90 D at one year (Table 5).

The mean keratometry value of the flatter meridian (Kmin) was 47.41 D (SD ± 3.50) (range: 45.12 - 56.12 D) prior to treatment (Table 6). At one month post-C3R, the Kmin value was 46.41 D (SD ± 7.53);

(p=0.001), and at 6 months post-C3R was 46.09 D (SD ± 7.53); p <0.0001) and at one year post-C3R was 45.91 D (SD ± 7.53); p <0.0001). This constant reduction in Kmin value from baseline throughout the follow-up period was statistically significant (p <0.0001).

The quantum of reduction of the mean Kmin value was found to be 0.11 D at one month, 0.44 D at 6months , and 0.63 D at one year follow-up, respectively (Table 6).

The mean average keratometry value (Kavg) at baseline was 50.45D (SD ± 3.75) (range: 47.25-57.62 D) (Table 7).There was reduction in Kavg value at one month post-C3R to 49.39 D (SD ± 8.03), and this reduction was statistically significant (p =0.002). On follow-up, the Kavg value was found to be further reduced to 49.01 D (SD ± 8.06), p <0.0001) at 6 months, and to 48.79 D (SD ± 8.06), p<0.0001) at one year post-C3R (Table 7). This reduction in Kavg value on follow-up was of statistical significance.

This study also recorded a reduction in the mean Kavg value compared to the baseline values during the follow-up, by a mean of 0.12 D at one month, 0.54 D at 6 months, and 0.77 D at one year post-C3R.

In this study, eyes that showed reduction in Kavg value by more than 0.5 D from the baseline, at the end of one year follow-up was considered improved and those eyes which showed reduction of less than 0.5 D were considered stable.

Thirty-four eyes showed improvement and 10 eyes remained stable at the end of one year follow-up in this study. Out of the 50 eyes in this study, 68% (34 eyes) showed improvement, whereas 20% (10eyes) remained stable. However an increase in the average K by less than 0.50 D was noted in four eyes. Two eyes that developed keratitis were excluded in this analysis. This indicated that C3R gives a beneficial response to arrest the progression of keratoconus overall.

And also, gradual reduction in the keratometric values was observed throughout the follow-up period post-C3R. All the visual and topographic indices observed over the period of one year after the procedure, showed a trend of either halting or partially reversing the disease process. The improvement was seen irrespective of the demographical factors such as age, gender and other pre-operative indices such as corneal thickness and the average keratometry value (Table 8).

In this study, two patients developed complications in the form of corneal ulcer within the first month of the procedure. Both the patients

were started on topical broad spectrum antibiotics. On further follow-up, one patient improved, but corneal scarring occurred with resultant steepening of average K values. On the other hand, in the other patient, the ulceration progressed necessitating therapeutic penetrating keratoplasty. Thus complications were noted in only two (4%) of 50 eyes in this study, and this was attributed to the use of bandage contact lens post- procedure.

This study found C3R to be an effective method for treating keratoconus providing good functional outcome in terms of visual and topographic parameters. These results were comparable to that of other studies so far conducted. However, a small sample size and short follow-up period were limiting factors of the current study.

Conclusion

CONCLUSION

In the current study, which aimed at evaluating the safety and efficacy of corneal collagen cross-linking with riboflavin (C3R) in keratoconus patients, the following conclusions were made.

The uncorrected visual acuity improved significantly from the baseline till one year of follow-up in all treated eyes. However, the best corrected visual acuity and the mean spherical equivalent showed a gradual improvement which was of statistical significance only at six months and one year of follow-up. This suggested delayed stabilization of the visual acuity, post-C3R.

This study also observed statistically significant reduction in the mean cylinder value throughout the follow- up period.

The topographic indices such as, the Kmax, Kmin and Kavg showed a gradual flattening in all the post-operative visits in a statistically significant manner.

The improvement was seen irrespective of the demographical factors such as age, gender and other pre-operative indices like corneal thickness and the average keratometry value.

All the visual and topographic indices observed over the period of one year after the procedure, showed a trend of either halting or partially reversing the disease process.

Thus, corneal collagen cross-linking with riboflavin is a simple, safe and an effective modality of treatment for progressive keratoconus with good success rate and minimal incidence of complications. However, longer follow-up is required for assessing the durability of this procedure and its long term side effects.

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Proforma

PROFORMA

Name: **MRD No:**

Age:

Sex:

Address:

Phone no:

Chief Complaints:

History:

Other associated systemic diseases:

Baseline parameters:

Eye	RE	LE
	Right eye	Left eye
Uncorrected Visual Acuity(UCVA)		
Best Corrected Visual Acuity(BCVA)		
DSPH		
DCYL		
AR		

Pre-operative data:

Examination	RE	LE
Lid and adnexa		
Cornea		
Anterior chamber		
Pupil		
Iris		
Lens		
Fundus		
IOP		
CCT		
K1		
K2		
Avg K		
Astigmatism		
CIM		

Indication:

Date of Procedure:

Post-operative data :

		Follow up after 1 month	Follow up after 6 month	Follow up after 1year
Vision	RE			
	LE			
RE	BCVA			
	DSPH			
	DCYL			
	AR			
LE	BCVA			
	DSPH			
	DCYL			
	AR			
K1				
K2				
Avg K				
Astigmatism				
CIM				
Complication				

Master Chart

MASTER CHART

MRD no.	Age	Sex	Eye	Pre UCVA	Pre BCVA	Dsph	Dcyl	Axis	SE	K1	K2	AVG K	USP	1 Month									6Month									1 year								
														UCVA	BCVA	Dsph	Dcyl	Axis	SE	K1	K2	Avg K	UCVA	BCVA	Dsph	Dcyl	Axis	SE	K1	K2	Avg K	UCVA	BCVA	Dsph	Dcyl	Axis	SE	K1	K2	Avg K
P695287	15	M	RE	5/60	6/18	-4	-2	180	-5	56.37	47.25	51.81	472	6/60	6/24	-3.5	-2	180	-4.5	55.87	47.15	51.51	6/36	6/18	-3	-2	180	-4	55.67	46.75	51.21	6/24	6/12	-2	-1.5	180	-2.75	55.27	46.5	50.885
P751409	12	M	RE	6/18	6/9	-2	-6	40	-5	54.5	46.75	50.625	449	6/18	6/9	-2	-5	30	-4.5	54.5	46.25	50.375	6/12	6/9	-2	-5	30	-4.5	54.25	46	50.125	6/9	6/6p	-1	-4	30	-3	55	45.81	50.405
P751409	12	M	LE	6/60	6/12p	-2	-4.25	170	-4.125	60.12	56.12	58.12	402	6/24	6/18	-2	-4.25	170	-4.125	59.82	56.12	57.97	6/60	6/18	-3.5	-3.25	160	-5.125	0	0	0	6/18	6/9	-1.5	-3.25	160	-3.125	0	0	0
P751931	21	M	RE	2/60	6/60	-9	-4.5	30	-11.25	58.75	51.37	55.06	436	6/60	6/36	-9	-4.5	30	-11.25	58.5	51.25	54.875	6/36	6/24	-8	-4	30	-10	57.75	51	54.375	6/24	6/18	-8	-3.5	30	-9.75	57.5	50.62	54.06
P761222	21	M	LE	6/24	6/12	-1.5	-5.5	180	-4.25	51.12	43.75	47.435	500	6/24	6/12p	-0.75	-6	155	-3.75	51.25	43.62	47.435	6/18	6/9	-0.75	-5.5	160	-3.5	51.12	43.6	47.36	6/12	6/9	-0.75	-5	160	-3.25	51	43.6	47.3
P771475	24	M	RE	6/60	6/18	-6	-3	155	-7.5	51	46.75	48.875	498	6/60	6/9	-6	-3	160	-7.5	50.37	46.12	48.245	6/60	6/9	-6	-3	160	-7.5	49.75	45.25	47.5	6/60	6/9	-6	-3	160	-7.5	49.5	45	47.25
P774377	25	M	RE	6/60	6/18	-1.5	-4	30	-3.5	56.25	48.62	52.435	424	6/36	6/12p	-2	-5	35	-4.5	56.12	48.62	52.37	6/36	6/9	-2	-5	35	-4.5	55.75	48.75	52.25	6/36	6/9	-2	-2	30	-3	55.87	48.25	52.06
P799854	11	M	LE	6/24	6/9	-2.5	-3	170	-4	53.87	47.25	50.56	482	6/24	6/9	-2.5	-3	170	-4	53.62	47.12	50.37	6/18	6/6p	-2	-3	170	-3.5	53.5	47.12	50.31	6/18	6/6	-2	-3	170	-3.5	53.25	47	50.125
P800768	19	M	LE	6/18	6/9	-3	-2	180	-4	50.72	46.87	48.795	456	6/18	6/9	-3	-2	170	-4	51.37	47.87	49.62	6/18	6/9	-3	-1.75	170	-3.875	50.75	47	48.875	6/12p	6/6p	-3	-1.5	170	-3.75	50.12	48.75	49.435
P801200	20	M	LE	5/60	6/9	-3.5	-3	115	-5	47.5	43.37	45.435	484	6/60	6/9	-3.5	-3	100	-5	47.45	43.35	45.4	6/36	6/9	-2	-3	100	-3.5	47.25	43.25	45.25	6/24p	6/6p	-1.5	-3	100	-3	46.75	42.85	44.8
P801200	20	M	RE	6/60	6/6	-3.5	-1	60	-4	45	42.75	43.875	502	6/36	6/6p	-3.5	-1	60	-4	44.85	42.65	43.75	6/24	6/6p	-3.5	-1	60	-4	44.5	42.5	43.5	6/12	6/6p	-3.5	-0.75	70	-3.875	44.12	42.5	43.31
P806124	21	M	LE	6/36p	6/24	0	-6	170	-3	57.37	45.87	51.62	451	6/24	6/18	-1	-5.5	170	-3.75	57.12	45.75	51.435	6/18	6/12	-1	-5.5	170	-3.75	56.75	45.5	51.125	6/12	6/6p	-1	-5	160	-3.5	56.5	45.35	50.925
P814272	24	M	LE	3/60	6/18	-4.5	-3	170	-6	53.37	50.62	51.995	456	6/60	6/9p	-4.5	-3	5	-6	54.75	50.25	52.5	6/60	6/12	-4.5	-3	180	-6	55	49.87	52.435	6/60	6/9	-4.25	3	180	-2.75	54.75	49.75	52.25
P815487	23	M	LE	6/60	6/12p	-6	-5	180	-8.5	59.87	50.25	55.06	454	6/60	6/12	-6	-5	180	-8.5	59.75	50.12	54.935	6/36	6/9	-5	-4	180	-7	59.5	50.12	54.81	6/24	6/9	-5	-4	180	-7	59.4	49.8	54.6
P815487	23	M	RE	6/9	6/6p	-1.25	-2.5	170	-2.5	52.75	45.62	49.185	475	6/9	6/6p	-1.25	-2.5	170	-2.5	52.6	45.5	49.05	6/9	6/6p	-1	2.5	170	0.25	52.5	45.52	49.01	6/9	6/6	-1	-2	180	-2	52.35	45.12	48.735
P820504	20	M	RE	5/60	6/9	-2.5	-3.5	20	-4.25	50.25	45.37	47.81	504	6/60	6/24	-2	-3.5	30	-3.75	49.85	45.15	47.5	6/36	6/9p	-2	-3	30	-3.5	49.5	44.85	47.175	6/36	6/6	-2	-3	40	-3.5	48.75	44.5	46.625
P820504	20	M	LE	5/60	6/9p	-2	-3	135	-3.5	48.5	44.37	46.435	512	6/60	6/18p	-2	-3	140	-3.5	48.25	43.75	46	6/36	6/9p	0	-3	150	-1.5	47.15	43.5	45.325	6/18	6/6p	0	-2.5	130	-1.25	46.75	43.5	45.125
P822730	13	M	RE	5/60	6/60	-10	-5	5	-12.5	60.25	56.12	58.185	446	6/60	6/18	-14	0	0	-14	60.12	55.75	57.935	6/36	6/18	-12	0	0	-12	59.25	55.12	57.185	6/36	6/12p	-14	-2	60	-15	58.72	55.12	56.92
P835473	24	M	RE	6/24p	6/9p	0	-2	140	-1	46.75	42.12	44.435	420	6/12	6/9	0	-2	140	-1	46.5	41.7	44.1	6/9	6/6	0	-1	140	-0.5	46.15	41.25	43.7	6/6p	6/6	0	-1	140	-0.5	45.75	41.25	43.5
P840452	15	M	LE	6/18p	6/9	0	-4	60	-2	57.75	49	53.375	490	6/18	6/9	0	-3.5	90	-1.75	57.5	48.75	53.125	6/12p	6/6p	0	-3.5	90	-1.75	56.75	48.25	52.5	6/9	6/6	0	-3	90	-1.5	56.5	47.85	52.175
P842462	20	M	LE	3/60	6/9	-8	-8	180	-12	60.37	52.25	56.31	454	6/60	6/9	-8	-6	180	-11	60.12	52	56.06	6/60	6/9	-8	-4	180	-10	59.85	51.75	55.8	6/60	6/9	-8	-4	180	-10	59.5	51.5	55.5
P842462	20	M	RE	5/60	6/9	-8	-3	15	-9.5	55.25	50.12	52.685	492	6/60	6/9	-8	-3	140	-9.5	55	49.85	52.425	6/60	6/9	-7	-3	140	-8.5	54.75	49.65	52.2	6/60	6/9	-7	-2.5	140	-8.25	54.5	49.35	51.925
P842546	15	M	RE	6/60	6/24	-3	-3	180	-4.5	52.25	45.62	48.935	511	6/60	6/18	-3	-3	180	-4.5	51.75	45.6	48.675	6/36	6/18	-3	-2	180	-4	50.83	45.61	48.22	6/24	6/12	-3	-1	180	-3.5	50.68	45.06	47.87
P842546	15	M	LE	6/24	6/12p	-1	2.75	150	0.375	52.5	45.12	48.81	521	6/24	6/12	-1	-2.5	150	-2.25	52.33	45.18	48.755	6/18	6/9p	-1	-2	150	-2	51.75	44.5	48.125	6/18	6/9	-1	-2	150	-2	51.5	44.5	48
P855286	13	M	RE	3/60	6/36	-7	-5	180	-9.5	57.37	49.37	53.37	421	6/60	6/12	-4.5	-3.5	180	-6.25	56.75	48.75	52.75	6/60	6/9p	-4.5	-3	180	-6	56.5	48.25	52.375	6/60	6/9p	-3.5	-3	20	-5	56.37	47.2	51.785
P840368	19	M	LE	6/24	6/12	-2	-2.5	160	-3.25	55.25	46.25	50.75	420	6/36	6/18	-2	-2.5	160	-3.25	54.85	45.85	50.35	6/18	6/12	-1.75	-0.5	150	-2	54.6	45.5	50.05	6/18	6/9	-0.5	-2	160	-1.5	54.5	45.5	50
P668964	15	F	RE	6/12	6/9	0	-3	180	-1.5	58.62	47.62	53.12	520	6/18	6/6p	-3	-1	180	-3.5	57.02	47.37	52.195	6/18	6/6	-3	-1	180	-3.5	56.67	47.2	51.935	6/18	6/6	-3	-0.75	180	-3.375	56.35	46.85	51.6
P685745	20	F	LE	5/60	6/36	-0.5	-3	180	-2	49.25	45.37	47.31	446	6/36	6/9p	-0.5	-3	180	-2	50.75	46.5	48.625	6/36	6/12	-0.75	-3	145	-2.25	49.62	45.62	47.62	6/18	6/9p	-0.5	-3	150	-2	48.37	44.25	46.31
P759128	25	F	RE	6/12p	6/9	-1.5	-6	35	-4.5	49.5	43.25	46.375	529	6/18	6/9p	-1.5	-5	30	-4	49.62	43.5	46.56	6/18	6/9p	-1	-4.5	30	-3.25	50.5	44	47.25	6/18	6/9	-1	-4	30	-3	50	42.87	46.435
P761900	25	F	RE	3/60	6/18	-5	-5	180	-7.5	48.75	44.12	46.435	506	6/60	6/36	-5	-4	180	-7	48.62	43.87	46.245	6/60	6/18p	-3.5	-4	180	-5.5	46	42.12	44.06	6/36	6/18	-3	-3.5	180	-4.75	45.8	42	43.9
P761900	25	F	LE	3/60	6/60	-3.5	-5	5	-6	49	44	46.5	507	6/60	6/24	-4	-3.5	5	-5.75	48.75	43.8	46.275	6/60	6/18	-4.5	-3	180	-6	48.37	43.62	45.995	6/36	6/12p	-3	-3	180	-4.5	47.85	43.32	45.585
P768581	19	F	RE	6/24p	6/12p	0	-2	60	-1	50.75	47.5	49.125	460	6/24	6/12	0	-1.5	70	-0.75	50.87	47.75	49.31	6/24	6/9	0	-2.5	40	-1.25	49.12	46.25	47.685	6/24	6/6p	0	-2	40	-1	49	46.12	47.56
P768581	19	F	LE	6/60	6/9p	0	-5	140	-2.5	53.75	48	50.875	439	6/60	6/12p	0	-3	130	-1.5	55.5	48.75	52.125	6/60	6/12p	0	-2.5	140	-1.25	52.12	47.5	49.81	6/36	6/9	0	-2	140	-1	52	47.25	49.625
P779440	23	F	RE	6/36	6/6p	0	-4.5	30	-2.25	49.12	44.62	46.87	485	6/18	6/9	0	-4	30	-2	49	44.52	46.76	6/12p	6/6p	0	-3	30	-1.5	48.92	44.52	46.72	6/18	6/6	0	-3	30	-1.5	48.87	44.82	46.845
P779492	13	F	RE	6/9	6/6	0	-2.25	170	-1.125	48	44	46	526	6/6	6/6	0	0	0	0	47.75	43.12	45.435	6/6	6/6	0	0	0	0	47.37	43.25	45.31	6/6	6/6	0	0	0	0	47.2	43.12	45.16
P781750	25	F	LE	6/60	6/24	-4	-2	140	-5	50	45.87	47.935	537	6/60	6/18	-4	-2	140	-5	49.75	45.5	47.625	6/60	6/18	-3.5	-1	140	-4	48.5	45.65	47.075	6/36	6/12	-3.5	-1	140	-4	48.5	45.55	47.025
P784968	25	F	RE	5/60	6/12	-0.75	-8.5	30	-5	53.25	45.12	49.185	460	6/60	6/12p	-2.5	-6	25	-5.5	54	45.25	49.625	6/9p	6/6p	-3.25	0	0	-3.25	52.5	44.87	48.685	6/9p	6/6p	-3.25	0	0	-3.25	52.25	44.75	48.5
P802352	20	F	RE	6/36p	6/18	-1.25	-3.5	60	-3	60.27	57.37	58.82	453	6/60	6/18	-1	-3	25	-2.5	60.12	57.1	58.61	6/60	6/18	-1	-3.5	35	-2.75	59.85	56.85	58.35	6/60	6/18	-1	-3	40	-2.5	59.7	56.87	58.285
P802352	20	F	LE	5/60	6/9p	-1.25	-3.5	130	-3	60.37	54.87	57.62	445	6/60	6/9	-1	-3.5	130	-2.75	60.15	54.25	57.2	6/36	6/9p	-1	-3	130	-2.5	59.8	54.15	56.975	6/24	6/9p	-1	-3	130	-2.5	59.65	54.1	56.875
P802434	15	F	RE	6/60	6/18p	-4	-4	10	-6	54.37	48.75	51.56	442	6/60	6/12	-3	-4	10	-5	54.25	48.5	51.375	6/60	6/9p	-3	-3.5	10	-4.75	53.87	48.25	51.06	6/36	6/6p	-3	-3	180	-4.5	53.37	48	50.685
P802434	15	F	LE	6/60	6/18p	-2.5	-4	160	-4.5	56.25	48.75	52.5	446	6/60	6/18	-3	-4	160	-5	55.75	48.5	52.125	6/36	6/12	-3	-3.5	160	-4.75	55.78	48.2	51.99	6/36	6/6	-3	-3	160	-4.5	55.5	47.9	51.7
P805990	24	F	LE	6/12p	6/6p	-2	-1	110	-2.5	47.62	45.25	46.435	472	6/18	6/6	-2	-1.75	110	-2.875	47.32	45.12	46.22	6/9	6/6	-2	-1.5	100	-2.75	46.82	45	45.91	6/9	6/6	-2	0	0	-2	46.52	44.72	45.62
P824626	19	F	LE	5/60	6/60	0	-5	140	-2.5	52.87	46.5	49.685	476	6/60	6/12	-4.5	-3	150	-6	52.37	46.5	49.435	6/60	6/12	0	-3	120	-1.5	51.37	44.62	47.995	6/60	6/12	-1	-3	130	-2.5	51.17	44.52	47.845
P831595	12	F	RE	6/60	6/12	-3	-4	20	-5	58.87	47.12	52.995	450	6/24	6/18	-2	-3	20	-3.5	58.75	47.87	53.31	6/24	6/12	-2	-3.5	20	-3.75	57.62	47.5	52.56	6/24	6/9p	-1.5	-3	40	-3	58.75	47.5	53.125
P831595	12	F	LE	6/60	6/24	0	-3	180	-1.5	57.62	47.37	52.495	462	6/36	6/18	0	-3	180	-1.5	58	47.5	52.75	6/36	6/12p	0	-2.5	180	-1.25	58.37	47.25	52.81	6/36	6/12	0	-2.5	120	-1.25	57.75	46.87	52.31
P842108	12	F	RE	6/24	6/9p	-0.5	-4.5	70	-2.75	48.62	44.12	46.37	531	5/60	6/60	0	0	0	0	0	0	0	6/60	6/18	-4	-3.5	30	-5.75	63.25	56.87	60.06	6/36	6/12	-3	-2.5	60	-4.25	63.12	56.87	59.995
P842108	12	F	LE	5/60	6/60	-8	-7	140	-11.5	57.25	51.12	54.185	491	6/36p	6/18	-5.5	-5	130	-8	57.15	50.87	54.01	6/24p	6/12	-5	-4	130	-7	57	50.87	53.935	6/24	6/9p	-3.5	-3	120	-5	56.25	51.62	53.935
P849401	24	F	RE	6/60	6/18	-2	-3.5	60	-3.75	52.12	46.12	49.12	470	6/60	6/12p	-2.5	-3.5	65	-4.25	49.8	45.75	47.775	6/36	6/12	-1	-3	45	-2.5	49.75	45.5	47.625	6/18	6/9	-1	-2.5	130	-2.25	49.25	45.25	47.25
P856276	20	F	RE	6/60	6/12	-3	-3	20	-4.5	55.87	48.75	52.31	454	6/60	6/18	-2	-3.5	30	-3.75	55.25	48.87	52.06	6/60	6/12	-2.5	-3.5	90	-4.25	55.87	49.12	52.495	6/60	6/12	-3	-3.5	90	-4.75	55.37	49	52.185
P856276	20	F	LE	6/60	6/9	-1	-2	130	-2	47.25	45.87	46.56	488	6/36	6/9	-1	-1.5	130	-1.75	46.85	45.57	46.21	6/18	6/6p	0	-1.5	130	-0.75	46.75	45.27	46.01	6/18	6/6	0	-1	130	-0.5	46.25	45	45.625